ABSTRACT

Title of Dissertation:	USING A HIGH ORGANIC-MATTER PERMEABLE REACTIVE BARRIER TO REMEDIATE TRICHLOROETHYLENE-CONTAMINATED GROUNDWATER AT THE BEAVER DAM ROAD LANDFILL
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Trichloroethylene (TCE) is an effective industrial degreaser and known carcinogen. It was frequently improperly disposed of and has become one of the most common groundwater and soil contaminants in the USA. Clean up continues to be difficult due to its physical and chemical properties. TCE and several of its degradation products were detected in the groundwater of the Beaver Dam Road Landfill (Beltsville, MD) at concentrations above their maximum contaminant levels (MCLs). The US Department of Agriculture-Agricultural Research Service together with the University of Maryland, College Park and BMT Designers and Planners designed a permeable reactive barrier, or biowall, to remediate the contaminated groundwater. A series of batch reactor studies were conducted at 12°C to examine biowall fill-material combinations including the effects of zero-valent iron (ZVI) and glycerol

amendments. Headspace samples were analyzed over the course of several months to monitor TCE degradation. An unamended, 4:3 mulch-to-compost combination was chosen based on no detectable TCE at the conclusion of the experiment. To increase the biowall degradation capacity, microbial infiltration and colonization of the structure were also studied. PCR, qPCR, and next-generation sequencing were used to survey the site's indigenous population for dechlorinating clusters. Numerous clusters were identified affirming the use of the native population for bioaugmentation efforts. The ability of the biowall to support said community was investigated by monitoring continuously-fed column reactors containing biowall material spiked with a commercially-available, surrogate population, with and without a 5 mg/L dose of ZVI. The groundwater-fed column sans ZVI had the greatest *Dehalococcoides* population and while ZVI without biostimulation did decrease the overall population, it did not cause a statistically significant difference. Thus, if ZVI were to be used as a future biowall amendment, biostimulation would not be required to maintain a dechlorinating population. A sacrificial carbon source may be necessary to slow the biological degradation of the biowall's organic fill-material. These findings will be utilized in future remediation and/or biowall expansion plans to fully employ the site's natural resources. The biowall was constructed in July 2013 containing the 4:3 mulch-to-compost ratio and has reduced the upstream TCE concentration by ~90%.

USING A HIGH ORGANIC-MATTER PERMEABLE REACTIVE BARRIER TO REMEDIATE TRICHLOROETHYLENE-CONTAMINATED GROUNDWATER AT THE BEAVER DAM ROAD LANDFILL

by

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Dedication

I wish to dedicate this body of work to the scientists and engineers working tirelessly to remediate the messes we have made in the past, to the future generations that will be tasked with cleaning up the messes we continue to make, and especially to Mother Earth- that this work and future endeavors bring some small measure of relief.

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Chapter 1: Introduction

Section 1: Background

TCE contamination

Trichloroethylene (TCE) is one of the most common groundwater and soil contaminants in the United States and a known carcinogen (Orth and Gillham 1996; Hendrickson et al. 2002; Zhang et al. 2015a,b; US EPA 2001). TCE has a drinking water maximum contaminant level (MCL) of 0.005 mg/L, or 5 ppb (US EPA, 2009). This compound is notoriously difficult to remediate as it is a relatively insoluble and hydrophobic compound, apt to form a dense non-aqueous phase liquid in aquifers, and also very volatile, readily escaping to the gas phase (Pant and Pant 2010). Its adsorption coefficient has been found to increase with increasing organic carbon content (Pant and Pant 2010).

Remediation protocol and planning

In general, initiating remediation protocols can be a labor and resource-intensive process. Ideally, site evaluation is a meticulous procedure as this step is instrumental to the successful remediation of the contaminated area of concern. Likewise, after the selection of a clean-up action, the development of an appropriate site monitoring plan is crucial. While scarce resources may limit the scope/breath of the monitoring plan by rationing efforts, these limitations may not necessarily hamper the remediation efforts if resources are wisely invested and success is clearly defined. The description of previous remediation efforts highlight some shortcomings that can occur in the field, namely during site characterization (i.e. hydrological mapping), development of the monitoring plan (i.e. spatial and temporal sampling), interpretation of data (i.e. water slug travel), and the length of time for which the remediation project will be monitored (AFCEE, 2008; FRTR, 2003; NATO/CCMS, 2001; Phillips et al. 2010; Vogan et al. 1999; Gilbert et al. 2013; GSI 2004; Lu et al. 2008; Wilkin et al. 2003).

The nature and timeline of the remediative action may dictate whether laboratory experiments or field trials are feasible and appropriate. However, it has been observed that the inclusion of experimentation is no guarantee that a remediation plan will be successful; that is, the success or lack thereof had more to do with the monitoring protocol and clearly defined goals (FRTR, 2003; Gilbert et al. 2013; GSI 2004; Lu et al. 2008; Vogan et al. 1999; Wilkin et al. 2003). In some instances, it could be interpreted that the individuals in charge misidentified the parameter of interest and instead measured something else, or misinterpreted their data to falsely conclude success. In a few cases, their remediation project was successful but they arrived at their conclusion incorrectly due to data mismanagement.

Section 2: Research objectives

The purpose of this work was to overcome the shortcomings of previous remediation efforts with careful planning, diligent monitoring, and conscientious analysis and interpretation. A permeable reactive barrier, or biowall, was installed to remediate (fully dechlorinate) trichloroethylene (TCE)-contaminated groundwater discovered at the Beaver Dam Road landfill in Beltsville, MD, USA. This site is a part of the

CERCLA, or Superfund, program which requires a coordinated effort by the US Environmental Protection Agency (US EPA) (as the overseer) and US Department of Agriculture-Agricultural Research Service (USDA-ARS) (active party). The University of Maryland-College Park and BMT Designers and Planners provided the research data to support the design of the remediation structure (biowall) and the monitoring plan.

A robust organization system was needed to both manage the contributing institutions and ensure/maintain a science-based approach regarding decisions about the biowall and the site's monitoring plan. Because science-based decisions were the first and foremost priority, it was vital that data be the foundation of the ongoing action plan to ensure that all decisions and actions came from a well researched and relevant position. This structure is intended to last many years (if not decades) and has been designed to protect both environmental and human health utilizing both abiotic and biotic degradation pathways. A hybrid model of active and passive adaptive management was used to organize communication between entities, manage the large site information databases, and allow for the incorporation of historic and new data into the design of the biowall and the site's monitoring plan. Scientific inquiry would be used to dictate process, methodology, and decision-making. The work presented here is organized under three main objectives.

Objective 1: Ascertain the biowall fill-material

The first research objective was to determine the composition of the biowall's fill-material (mulch/compost composition, inorganic/organic material ratio) in order to provide recommendations to BMT Designers and Planners. Mulch, compost, and concrete sand were selected as the major construction components, while zero-valent iron (ZVI) and glycerol were investigated as possible degradation aids. The null hypotheses tested are as follows:

H_{0-1}: An unamended 4:3 mulch-compost mixture will not provide the ideal environment for trichloroethylene degradation (40% inorganic material, 60% organic material).

H_{0-2}: No intervention is needed to maintain stable internal reactor conditions; pH, total alkalinity, and redox conditions will change over time but not interfere with TCE dechlorination.

H_{0-3}: No significant difference in the TCE degradation profile between reactors dosed with the zero, low, and high levels of ZVI/L

H_{0-4}: No significant difference in the degradation profile of TCE with respect to the dose of glycerol added to the reactors (zero, low, high dose).

To address these hypotheses, batch reactors were constructed in triplicate (including controls) to mimic the contaminated groundwater conditions, including anaerobic, saturation, and temperature conditions. The degradation of trichloroethylene was monitored at regular intervals by sampling the headspace of the reactors and using established analytical methods to determine the phase-related concentrations. At the conclusion of the experiment, the pH, alkalinity, and redox condition of each reactor was measured under controlled, anaerobic conditions. The mixture that was to be implemented in the field had to fulfill two requirements: first, the mixture degraded TCE to below its maximum contaminant level (MCL), a parameter used to determine a "safe" concentration in drinking water, and second, the mixture did not produce other toxic degradation products above their respective MCLs.

Additional post-batch experiments included measuring the total organic/inorganic carbon content to understand fill-material degradation and the necessity of including a sacrificial carbon source to support biological function (i.e. glycerol or other); measuring select organic/inorganic compounds to monitor material aging; constructing an anaerobic, room-temperature plug-flow reactor to better mimic the constant groundwater flow; and lastly, measure the aerobic release of VOCs from the batch reactor fill-material to understand the toxicity of the spent fill-mixture that will have to be removed from the biowall and disposed of at some point in the future. Findings from objective 1 are presented in Chapter 2, in the form of a manuscript submitted to *Environmental Science and Pollution Research*.

Objective 2: Ascertain the presence of *Dehalococcoides* spp. at the Beaver Dam Road landfill and investigate the broader microbial population

The second research objective was to determine if *Dehalococcoides* spp. were present at the biowall site, and if there is a correlation between their presence and/or population size and the concentration of trichloroethylene. Previously, TCE was thought to be resistant to biodegradation until Maymó-Gatell, et al. (1997) discovered *Dehalococcoides ethenogenes* strain 195 (reclassified as *Dehalococcoides mccartyi* strain 195), capable of fully and completely dechlorinating tetrachloroethylene (PCE), (Hendrickson et al. 2002; Maymó-Gatell et al. 1997; Loeffler et al. 2013). Along with the discovery of several strains of this bacterium, Lee, et al. (2006) determined that expression of particular functional genes responsible for reductive dechlorination could be used as a biomarker to determine activity of the bacteria, though not as a method for correlating contaminant concentration and dechlorination rate.

Here, the null hypotheses are as follows:

$H_{0\text{-}1}$: *Dehalococcoides* spp. are not present in the tested site soil

$H_{0\text{-}2}$: There is no correlation between the concentration of *Dehalococcoides* spp. and the concentration of trichloroethylene;

$H_{0\text{-}3}$: None of the isolates from the contaminated soil cores contain any of the known dechlorination genes, such as *tceA*, *vcrA*, or *bvcA*.

To address this research objective and hypotheses, soil samples were collected from locations upstream of the biowall structure in areas known to contain a high and moderate TCE concentration as well as from an adjacent field with a “zero” TCE concentration. DNA was extracted from the soil samples and screened for *Dehalococcoides* spp. and select functional genes using PCR and real-time PCR (qPCR). Previously established analytical methods were used to search for a statistical correlation between *Dehalococcoides* presence and TCE concentration.

Later it was decided to expand the scope of the second objective to include an investigation of the larger microbial community. Additional soil samples were collected from the biowall structure itself in locations corresponding to the original soil samples previously gathered. Next-generation sequencing was used to identify known groups of microbes beneficial for the degradation of TCE, DCE, and VC as well as investigate the migration/infiltration of the soil microbial community into biowall material. This information is to be used to inform future bioaugmentation efforts to target specific individuals or groups. The findings from this work are presented in Chapter 3 in the form of a manuscript submitted to the *Journal of Applied Microbiology and Biotechnology*.

Objective 3: Ascertain the need for biostimulation of the biowall in order for it to support a mixed *Dehalococcoides* population

Finally, the third project objective was to determine if the biowall would be a habitable environment for *Dehalococcoides* and/or other TCE-degrading communities to flourish, or if bio-stimulation and/or -augmentation would be necessary to maintain these populations. Because ZVI was considered as a potential amendment to the fill-material, it was important to determine if its presence would negatively influence the microbial populations. Previous studies suggest that the addition of ZVI to a flow-through reactor may sorb and/or inactivate bacteria (Gu et al. 1999; Wilkin et al. 2005; Ingram et al. 2012). Likewise, nanoparticles of ZVI have demonstrated an increase in microorganism toxicity not consistently observed with larger particles (Lee et al. 2008; Diao and Yao 2009; Chen et al. 2011). Another study added ZVI to a sand filter for decontaminating irrigation water prior to field application. Here, the sand filters with ZVI had significantly lower levels of *E. coli* in the effluent compared to the sand filters sans ZVI though it was unclear if the bacteria trapped in the ZVI-amended sand filter were killed or merely deactivated (Ingram et al. 2012).

The null hypotheses tested for this objective were:

$H_{0\text{-}1}$: The biowall will not require the addition of a nutrient source (modified RAMM media) as biostimulation in order to support a desired population of specialized microorganisms (measured as total *Dehalococcoides*)

$H_{0\text{-}2}$: The addition of a low ZVI dose to the biowall material will not significantly affect the *Dehalococcoides* population.

To address this research objective and hypotheses a series of flow-through reactors containing SDC-9 (model community) and biowall fill-material with or without a low dose of ZVI were constructed. Each reactor was continuously fed with either TCE-spiked, nitrogen-sparged groundwater or TCE-spiked, nitrogen-sparged nutrient broth (made with groundwater) in an anaerobic, room temperature environment. At the termination of the experiment, the columns were dissected and the fill-material underwent DNA extraction to measure the total *Dehalococcoides* population as well as the prevalence of select functional genes. Unfortunately, the lack of sampling ports along the body of the reactors prevented measurement of the TCE-degrading capability under these more realistic environmental conditions. The findings from this work are also presented in Chapter 3 as part of the manuscript submitted to *Journal of Applied Microbiology and Biotechnology.*

Objective 4: Develop a management system to organize and direct a multi-agency, multi-faceted Superfund remediation project

By using a science-based approach to test the various hypotheses, it was possible to formulate a well thought-out remediation and long-term monitoring plan that could account for site maturation, evolution of the contaminants of concern, and changes to the remediation priority. This was possible in part by utilizing adaptive management to organize and oversee the various research components and interested parties (EPA, USDA-ARS, BMT Designers and Planners, University of Maryland). We reflect on the exercises undertaken to employ both passive and active adaptive management systems in Chapter 4 as a manuscript submitted to the *Journal of Environmental Management*.

Chapter 2: Using a high-organic matter biowall to treat a trichloroethylene plume at the Beaver Dam Road landfill

Opening remarks on biowall structure and function

Biowalls, or permeable reactive barriers (PRBs), are in-ground trench-like structures that trap and degrade dissolved pollutants within the wall as water passes through. They are strategically placed to take advantage of surrounding hydraulic conditions and are usually built at the leading edge of the contamination plume. Biowalls are implemented as a less expensive, “greener” alternative to the more traditional solutions for dealing with contaminated soil and groundwater issues (e.g. pump-and-treat; soil burning and replacement; air stripping). Not only are these solutions more environmentally destructive and/or cumbersome, but they provide temporary solutions to areas where the contamination source is hard to find or difficult to reach. Biowalls provide on-going protection for the life of the wall and require little to no maintenance. Construction issues may include subsidence if improper support materials are used, flooding of the area behind the wall if hydraulic conductivity is too low (insufficient drainage), or flooding of the surrounding area if hydraulic conductivity is too high (water passes through without impediment).

While biowall technology can be widely applied and generously defined, it is an engineered structure that requires tailoring to the contaminant of concern, the site/environmental conditions, and most importantly the remediation (end) goals. As a structure that is incorporated into the soil, it can be years before the structure functions as it was intended. This is in part due to naturally occurring processes such as subsidence of the structure and settling, but also the working relationship between abiotic and biotic factors; in some instances, biotic infiltration of the biowall is actively discouraged while in others, biotic interference is either passively tolerated or actively encouraged by the engineers (AFCEE 2008). The addition of electron donors and acceptors to the biowall materials can stimulate microbial activity, thus generating minerals via microbial reduction of iron and sulfate, and thereby encouraging microbially-mediated reductive dechlorination, a predominant pathway for tetrachloroethylene (PCE) and TCE dechlorination and an important consideration during biowall design (Dong et al. 2009; Maymó-Gatell et al. 1997).

Anaerobic degradation products from the biological breakdown of TCE include *cis*- and *trans*-dichloroethylene (DCE), vinyl chloride (VC), and ethene whereas abiotic degradation of TCE typically follows a β-elimination pathway that forms acetylene, which may further degrade to ethene and/or ethane (Farrell et al. 2000; He et al. 2008). MCLs for these contaminants are listed in Table 1, below.

Field reaction rates have shown dependence on the abundance of dechlorinating bacteria, soil properties, and mass loading of reactive materials (Dong et al. 2009). Observations and measurements made by Magnuson et al. (1998) showed that the reduction-rate order proceeded as cis-dichloroethylene (DCE) > 1,1-DCE > TCE > trans-DCE > vinyl chloride (VC) contrary to the expected order based on free energy calculations (TCE > VC > DCEs). This difference implies that kinetic factors control the fitness of each product for reductive dechlorination rather than thermodynamic factors (Magnuson et al. 1998). As for geochemical factors such as weakly- and strongly-bound Fe(II), acid-soluble sulfur, and chromium-extractable sulfur (CrES), there have not yet been reports regarding their influence over the transformation kinetics of PCE and TCE (Dong et al. 2009).

Brief history of zero-valent iron (ZVI)

ZVI is a commonly used, valuable material in TCE degradation due to its ability to reductively dechlorinate a variety of chlorinated organic compounds without the help of additional materials (Farrell et al. 2000; Orth and Gillham 1996). ZVI is a very strong reducing agent (E_h = -440 mV) and degradation generally follows either hydrogenolysis or a β-elimination pathway (Chen et al. 2011). Though β-elimination has proven to be the dominant pathway, hydrogenolysis appears to garner greater attention (Arnold and Roberts 2000; Farrell et al. 2000; Schäfer et al. 2003). During β-elimination, TCE is transformed to ethane or ethene via a series of intermediates while during hydrogenolysis, intraspecies competition plays a large role in degradation rate (Gavaskar 1999). When both TCE and *cis*-dichloroethylene (*cis*-DCE) were mixed with ZVI, there was almost no effect to the degradation rate of TCE while the degradation rate of *cis*-DCE was significantly decreased when compared to an earlier trial where only one contaminant at a time was mixed with ZVI (Schäfer et al. 2003). In the same study, the degradation rate of *trans*-DCE in the presence of *cis*-DCE or acetylene decreased by 50% and 90%, respectively, though acetylene (formed during β-elimination) had no change in reaction rate when the concentration of other species changed. In separate studies, at high (>200 μM) mixed solution concentrations of TCE and *cis*-DCE, degradation was zero-order, while at low (<0.008 μM) concentrations, degradation followed first-order kinetics (Scherer and Tratnyek 1995; Wüst et al. 1999). Different exposure studies have found that effectiveness of ZVI is due to both sorption and chemical degradation. In Arnold and Roberts (2000) various PCE degradation products were exposed to (a zero-to-low carbon content) ZVI either singly or in mixtures; the less chlorinated daughter products were more reactive to ZVI than the more highly chlorinated parents. In Farrell et al. (2000), as the surface of ZVI became less reactive, the production of more fully reduced compounds decreased. In Orth and Gillam (1996), low solution concentrations of TCE (or other analytes) were correlated with high surface sorption until complete dechlorination occurred.

Field studies involving ZVI have focused on the formation of pyrite (FeS_2) and the presence of organic matter as both have been found to interfere with the TCE degradation rate and the ability of ZVI to function at maximum capacity (Farrell et al. 2000; He et al. 2008). Soils naturally containing iron compounds experience iron (II) sulfide (FeS) transformation to FeS_2; FeS is the mineral responsible for the prompt breakdown of TCE and as its quantity decreases, the rate of TCE degradation also decreases (He et al. 2008).

Brief history of glycerol use

Glycerol ($C_3H_8O_3$), also known as glycerin, is a trihydroxy sugar alcohol that is miscible in both water and alcohol, and is hygroscopic (Tan et al. 2013). It is an abundant byproduct of biodiesel manufacturing, typically consisting of 20% water in this crude form. It has a low level of toxicity and is a viscous liquid with a density of 1.26 g/mL (20°C) and a log K_{OW} -1.76 (Hughes and Robertson 2002). In its pure form, it can be found in pharmaceuticals and used as an emollient, solvent, or sweetener (Tan et al. 2013). Glycerol is readily biodegradable under both aerobic and anaerobic conditions (Hughes and Robertson 2002). It is relatively inexpensive and widely available, is non-flammable and less toxic than methanol and acetate, two additives that are commonly used as electron donors for reductive dechlorination. For these reasons, glycerol was considered as an amendment in the experimental biowall design as opposed to methanol or acetate.

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Abstract

Trichloroethylene (TCE) is a highly effective industrial degreasing agent and known carcinogen. It was frequently buried improperly in landfills and has subsequently become one of the most common groundwater and soil contaminants in the USA. A common strategy to remediate TCE-contaminated sites and to prevent movement of the TCE plumes into waterways is to construct biowalls which consist of biomaterials and amendments to enhance biodegradation. This approach was chosen to contain a TCE plume emanating from a closed landfill in Maryland. However, predicting the effectiveness of biowalls is often site specific. Therefore, we conducted an extensive series of batch reactor studies at 12 °C as opposed to the typical room temperature to examine biowall fill-material combinations including the effects of zero-valent iron (ZVI) and glycerol amendments. No detectable TCE was observed after several months in the laboratory study when using the unamended 4:3 mulch-to-compost combination. In the constructed biowall, this mixture reduced the upstream TCE concentration by approximately 90% and generated ethylene downstream, an indication of successful reductive dechlorination. However, the more toxic degradation product vinyl chloride (VC) was also detected downstream at levels approximately ten times greater than the maximum contaminant level. This indicates that incomplete degradation also occurred. In the laboratory, ZVI reduced VC formation. A hazard quotient was calculated for the landfill site with and without the biowall. The addition of the biowall decreased the hazard quotient by 88%.

Introduction

Trichloroethylene (TCE) is a highly effective and efficient industrial degreasing agent and solvent, and because of its historically poor disposal, it is also one of the most common groundwater and soil contaminants in the USA (Orth and Gillham 1996; Hendrickson et al. 2002; Zhang et al. 2015). Discharges from metal degreasing sites and other factories are the most common sources of TCE (US EPA 2001). TCE is of concern because it is a known carcinogen and, with prolonged exposure, can increase the chances of liver, lung, and nervous system issues (Chiu et al. 2013). In addition, several degradation products of TCE, such as vinyl chloride (VC), are more toxic than TCE (US EPA 2009).

While present in a large portion of sites around the USA, cost-effective remediation of TCE-contaminated sites is difficult due to its volatility (vapor pressure = 9700 Pa; Chiao et al. 1994), low water solubility (water solubility = 1.1 g/L; US EPA 2016a), and high density (1.46 g/cm^3; Jacoby et al. 1998), leading to its tendency to form a dense non-aqueous phase liquid layer in aquifers (US EPA 2016a; Pant and Pant 2010). Studies have also shown that its adsorption coefficient (K_d) is dependent on the soil organic carbon content (f_{OC}) and its organic carbon–water partitioning coefficient (K_{OC}) ($K_d = K_{OC} \times f_{OC}$); the larger the f_{OC}, the greater the K_d (Lee et al. 2007; Pavolostathis and Jaglal, 1991; Pant and Pant 2010; Poulsen et al. 2000). Thus, complete and effective TCE remediation strategies remain elusive.

Under biologically active, anaerobic conditions, TCE will undergo reductive dehalogenation (principal pathway) and form *cis*- and *trans*-dichloroethylene (DCE), VC, and ethylene (Tandoi et al. 1994; Maymó-Gatell et al. 1999; Wu et al. 1998; Pant and Pant 2010). Many different members of the microbial community can contribute to this degradation pathway, though DCE and VC can accumulate, and subsequent conditions may limit continued degradation to the goal of complete dechlorination (Bradley and Chapelle 1998; Maymó-Gatell et al. 1999; Yu et al. 2005; Atashgahi et al. 2017). Abiotic degradation of TCE typically follows a β- elimination pathway that forms acetylene, which may further degrade to ethylene and/or ethane, as well as a host of other compounds (Farrell et al. 2000; He et al. 2008; Arnold and Roberts 2000). It is expected that these pathways can co-exist in open environmental systems.

In 1998, TCE was identified in the groundwater system surrounding the Beaver Dam Road landfill at a concentration several orders of magnitude above its maximum contaminant level (MCL) of 5 ppb, posing a potential threat to human and environmental health (USDA-ARS 2012; US EPA 2009). Located in Beltsville, Maryland, USA, the grass-covered land- fill and a portion of the surrounding semi-wooded wetland area occupying approximately 1 ha were enrolled in the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) program. The landfill was used for the disposal of construction debris and demolition rubble from the 1940s to the 1980s before it was capped in 1990 in adherence with the existing state regulations governing such facilities (BMT Entech Inc. 2008, 2009; USDA-ARS 2012). Subsequent site investigations, however, determined that a polluted-groundwater plume, approximately 200 m wide by 140 m long, emanated southeast of the landfill and was flowing at an estimated 12.5 m year^{-1} (USDA-ARS 2009; BMT Designers and Planners Inc., 2016). As a result, remediation was required. After consultation with the US EPA and careful consideration of the site conditions and TCE properties, a permeable reactive barrier, or biowall, was chosen for TCE containment and remediation at this site (Niño de Guzmán et al. 2017).

Biowalls are a customizable, low-maintenance, groundwater remediation technology which has been utilized for several decades for a wide variety of contaminants. Akin to in-ground filters, dissolved groundwater contaminants are sorbed and degraded within the fill materials as water flows through the structure. A biowall does not directly address the contaminant source, but is instead placed downstream of it to manage the discharged contaminant(s) and to prevent them from migrating outside of a designated area, thus minimizing risk.

Typically, these structures are used when the source cannot be remediated directly, the site requires highly effective but minimally invasive action, and/or the emission timeframe is either very long or unknown since biowalls have inherently long lives. Many studies have discussed the design parameters and materials (fill, electron donor, carbon source, or other additives) to consider when building a structure compatible with the site and contaminants (Ahmad et al. 2007; Erto et al. 2011; Tratnyek et al. 1997; Yang et al. 1995; AFCEE 2008; Ozturk et al. 2012). In the current study, the organic portion of the landfill biowall was composed of mulch and compost with sand and gravel acting as stabilizing materials. The hemicellulose and cellulose content of mulch/compost fill materials is one factor to consider when deciding how often a biowall may need to be replenished as these components are the first to be broken down during the natural degradation process (Ahmad et al. 2007). The lignin content may also be useful to predict structure hardiness as this material is relatively resistant to degradation, particularly in anaerobic conditions. In certain circumstances where biological activity is desired, it may be necessary to provide an easily degradable carbon source to maintain the microbial community as the complex carbon compounds in the wood and compost of the biowall fill materials may degrade too slowly to keep up with demand. Conversely, a supplemental carbon source may be necessary as a “sacrificial” supply to slow the biological degradation of the fill material.

Zero-valent iron (ZVI) has been widely used to promote TCE degradation as it is a highly reduced material and amenable to hydrogen ion generation (Farrell et al. 2000; Wilkin et al. 2003; Phillips et al. 2010; Burris et al. 1995; Kouznetsova et al. 2007). While the uses and reaction mechanisms of ZVI shavings versus nanoparticles differ, overall, research has shown that as ZVI ages and deposits form on the particle surfaces due to constant contact with groundwater and the surrounding environment, dechlorination rates and pore space decrease over time (Weber 1996; Farrell 1996; Wilkin et al. 2003; Liu et al. 2005; Kouznetsova et al. 2007). Therefore, the use of this material may require occasional reintroduction into the biowall structure in order to preserve high dechlorination rates. It should also be noted that ZVI has demonstrated antimicrobial effects, and in instances where a high microbial population is favored, this material may war- rant further investigation (Lee et al. 2008; Ingram et al. 2012; Yang et al. 2013; Zabetakis et al. 2015). Another drawback to this material is cost.

Methanol and acetate are two biowall additives commonly used as electron donors for reductive dechlorination, but both can be toxic (Hughes and Robertson 2002; Freeborn et al. 2005; NIOSH 2015). Therefore, glycerol ($C_3H_8O_3$) was considered as a possible biowall addition. It is readily biodegradable under both aerobic and anaerobic conditions, is non-flammable, and is non-toxic. Additionally, glycerol is relatively inexpensive and a widely available resource (da Silva et al. 2009). Several studies have used glycerol to provide the native soil consortium with a potential electron donor and an alternative carbon source in the interim period where compost and mulch degradation rates are slow (Qatibi et al. 1998; da Silva et al. 2009; Viana et al. 2012; Emde et al. 1989; Bertolino et al. 2014).

Previous studies have combined some of these materials to remediate TCE groundwater contamination and other similar environmental issues. The success of these efforts has been a function of the physical and chemical properties considered during the laboratory bench-scale experiments, as well as those measured in the field (Ahmad et al. 2007; Erto et al. 2011). Equally important, however, has been the monitoring plan to track the efficacy and maturation of the structure. In some case studies, it was determined that the groundwater flow rate, sampling location, and data interpretation were incompatible; in others, the monitoring period was not sufficiently long or detailed to fully appreciate the impact of the remediation plan (Federal Remediation Technologies Roundtable (FRTR) 2003; FRTR 2010; Groundwater Services, Inc. (GSI) 2004; Phillips et al. 2010; Tratnyek et al. 1997; Vogan et al. 1999; Liu et al. 2005; Gilbert et al. 2013; Wilkin et al. 2003).

Here, we describe a series of batch experiments conducted prior to and concurrent with the construction of the biowall in July 2013, to (1) determine the most effective mulch/compost composition of the biowall fill material, (2) study the effects of including ZVI shavings and/or glycerol with the bulk material to degrade TCE, and (3) identify the TCE degradation profiles that could be expected under the anaerobic, groundwater conditions at the site. To best mimic the environmental conditions of the landfill, these experiments were conducted anaerobically at the relevant 12 °C under anaerobic conditions instead of at room temperature, as has typically been done. The biowall monitoring plan was diligently adjusted to consider new data as well as emerging technology.

Materials and methods

Batch reactor systems

One-liter glass batch reactors were assembled in triplicate using ten different mixtures of compost, sand, ZVI, and glycerol. Each replicate group (group 1, group 2, and group 3) was constructed on a different day to stagger the headspace measurements. All compost and mulch used in the laboratory experiments were obtained from the landfill area and stored outdoors at the nearby USDA composting facility. The compost consisted of a blend of 10% food residual compost and 90% leaf compost generated at the compost facility. The mulch was generated from a wide variety of trees, mainly tulip poplar, removed at the site, with a diameter 2.5 cm or less. Two organic fill mixtures were investigated: a 1:1 mulch/compost (M1C1) blended with a 40%:60% inorganic-to-organic ratio (Ahmad et al. 2007; Federal Remediation Technologies Roundtable, 2003) and a 4:3 mulch/compost (M4C3) blended with a 30%:70% inorganic- to-organic ratio, recommended by BMT Designers and Planners

(Arlington, VA), the engineering company contracted to construct and install the biowall structure.

The inorganic fraction was autoclaved (121 °C, 35 min) sand, which also served as the negative control reactor. ZVI shavings of aggregate size 8/50, density 6.62 g/mL (Peerless Metal Powders and Abrasive, Detroit, MI) were added to select reactors at three dose levels, 0, 5, and 10 mL/L. Glycerol (ACROS Organics, New Jersey) was added to select reactors at three dose levels, 0, 10, and 30 mL/L. Soil for the control (or "do-nothing" condition to the site) reactors was collected from an uncontaminated area close to the landfill using a Geoprobe (Geoprobe Systems, Salina, KS). The soil cores were photographed, wrapped in packing plastic, placed on ice, and transported to the laboratory, then stored at 4 °C.

The mulch–compost mixture was added first to each 1-L reactor [M1C1 average weight 560 g ± 11 g (SD); M4C3 average weight 500 g ± 0.1 g (SD)] before the addition of ZVI [low dose (Fe5) = 6.1 g, high dose (Fe10) = 12.2 g] and/or glycerol [sterile pipette, low dose (G10) = 5 mL, high dose (G30) = 15 mL]. Contents were thoroughly mixed and 250 mL of sterile deionized water was added. The reactors were capped with Silonite™-coated stainless steel lids outfit- ted with male micro-QT valves (Entech Instruments Inc., Simi Valley, CA) and allowed to stabilize for 48–72 h. Each reactor was connected to a vacuum pump for at least 10 min to evacuate oxygen and then re-pressurized to 16 psi with ultra-high- purity N_2 (Airgas, Allentown, PA) using an Entech 4600A Dynamic Diluter (Entech Instruments Inc., Simi Valley, CA). A slightly positive pressure was used to distinguish between reactor leaks and gas generation. Reactors were spiked with 700 µg of TCE in 50 mL sterile deionized water through the micro-QT valve. To mimic groundwater conditions, reactors were kept at 12 °C (± 2 °C) for approximately 160 days. No active steps were taken to regulate alkalinity, pH, or to encourage the microbial growth of any organisms naturally present in the mulch or compost materials.

At the conclusion of the experiment, the pH and redox potential of each reactor were measured in triplicate (Thermo Scientific, Waltham, MA) (groups 1 and 2) inside an N_2-only anaerobic glove box (Coy Lab Products, Grass Lake, MI) to protect the established anaerobic environment. A portion of water was drained from the reactors, nitric acid- fixed, and analyzed using a Shimadzu ICPE-9000 multi-type ICP emission spectrometer (Shimadzu, Columbia, MD). Approximately 10 g of solids was freeze-dried and ground with a mortar and pestle, then assessed for total and inorganic carbon content in triplicate using a Shimadzu SSM-5000A (Shimadzu, Columbia, MD) solid sample analyzer. Group 3 reactors were set aside and used for a supplementary set of aerobic experiments, not described here.

Analytical methods

Weekly headspace sampling was initiated following a 3-day equilibration period after spiking with TCE. A gas-tight syringe (VICI Precision Sampling Inc., Baton Rouge, LA) was used to collect a 600-µL headspace sample. Each reactor was sampled in triplicate. Samples were analyzed using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (GC-FID) (Agilent Technologies, Santa

Clara, CA) and a Supelco SLB-5ms (30 m × 0.25 mm × 0.25 μm) fused silica capillary column (Supelco, Bellefonte, PA). Instrument settings were as follows: oven 40 °C to 75 °C at 20 °C/min, hold 1.75 min, and then to 150 °C at 45 °C/min, hold 30 s; inlet temperature = 200 °C and flow (He) = 3.0 mL/min, splitless; detector at 280 °C, H_2 = 40 mL/min, air = 350 mL/min, makeup N_2 = 30 mL/ min; these settings were based on an amalgam of various in-house trials and an initial conversation with Agilent Technologies. Samples were quantified for acetylene; ethylene; 1,2-dichloroethylene (DCE); cis-1,2-DCE; trans-1,2-DCE; trichloroethylene (TCE); and vinyl chloride (VC). Acetylene and ethylene co-eluted as did all of the DCE isomers (Online Resource 1). Method detection limits (MDLs) and limits of quantitation (LOQs) were determined (Table 1). Standards were prepared from a custom gas blend composed of 100 ppm each of trichloroethylene, cis-1,2-dichloroethylene, VC, and ethylene, balance nitrogen (RESTEK Corporation, Lancaster, PA); a separate 100 ppm standard was used for acetylene preparation (RESTEK Corporation, Lancaster, PA). An eight- point calibration curve was prepared ranging from 0 to 100 ppm.

Table 1-1: Maximum contaminant levels (MCLs), method detection limits (MDLs) and limits of quantitation (LOQs)

Analyte	Acronym	MCL (ppb)	MDL (ppm)	LOQ (ppm)
Trichloroethylene	TCE	5	0.88	3.57
***cis*-1,2-Dichloroethylene**	DCE	70	0.82	3.46
Vinyl chloride	VC	2	0.13	1.94
Ethene/Acetylene			0.58	3.05
Acetylene			0.16	0.77

A TCE dissipation rate constant, k_{TCE}, was calculated from a single-phase decay model,

$$C_g = (C_{g0} - Plateau) \times \exp(-k_{TCE}\, t) + Plateau \quad (1)$$

where C_g is concentration of TCE in the gas phase and *Plateau* refers to the asymptotic value of each data set (GraphPad Prism software, version 5.01; GraphPad Software, Inc., La Jolla, CA). If a background concentration has been pre-screened from the data set, this Plateau value is zero. Dissipation and production rates were calculated for DCE (k_{DCE}) and VC (k_{VC}) using linear regression models ($y = mx + k_z$), where m is the y-intercept and k_z is the slope of the line.

A dimensionless Henry's constant (H = 0.181; 1 atm, 12 °C) for TCE was calculated from the equation published in Heron et al. (1998). Separate smaller reactors were constructed to determine an approximate adsorption coefficient (K_d) for the M4C3 mixture following the method outlined in Lee et al. (2007).

Statistical analysis

GraphPad Prism (GraphPad Prism soft- ware, version 5.01; GraphPad Software, Inc., La Jolla, CA) was used to run a one-way ANOVA on each batch reactor group to determine if there was a statistical difference between the contents of each reactor based on TCE degradation data. The ANOVA was combined with the Kruskal–Wallis test and followed by Dunn's post test. The Kruskal–Wallis test is a non-parametric test that com- pares three or more unmatched data sets to determine if they have come from identical populations (GraphPad Prism 2017; McDonald 2014). Dunn's post test compares the data sets to each other to determine if the data come from populations with identical distributions and if any differences between pairs are due to random sampling (GraphPad Prism 2017).

Site description and field measurements

With the cessation of disposal activities at the landfill, a synthetic landfill cap was placed over the landfill, covered with soil, and seeded to provide vegetative cover. The landfill lies approximately 18 m south of Beaver Dam Road and is surrounded by a semi-wooded area to its east and south; a cultivated research field sits to the west. Further south lays an un- named tributary and wetland, where the groundwater flows in a southeasterly direction toward the unnamed tributary. In July 2013, the biowall was installed by BMT in conjunction with a subcontractor. The dimensions of the in-ground structure are approximately 305 m long, 0.7 to 0.8 m wide, and 5.5 to 7 m deep. The fill material used in the biowall is composed of 40% mulch, 30% compost, and 30% sand by volume.

In accordance with the Record of Decision to remediate the site using a biowall, a groundwater monitoring program was developed by BMT Designers and Planners. Groundwater from ten biowall wells (BWs), six transect wells (TWs), and nine remedial investigation wells (MWs) at the study site (Fig. 1) were sampled either biweekly or quarterly beginning in November 2013 by BMT Designers and Planners. The biweekly measurements at the BWs included physical parameters (dissolved oxygen, redox potential, temperature, pH, salinity, turbidity, and specific conductivity) using a fixed volume purge method with a Horiba U-52 Multiparameter

meter (Horiba Ltd., Alvin, TX) and a YSI ProODO Optical Dissolved Oxygen O meter (YSI Inc., Yellow Springs, OH). Three well volumes were purged in order to sample water representative of the groundwater conditions using highflow evacuation with dedicated bailers (ASTM 2012). It should be noted that when this method is used in shallow wells, some parameters (e.g., DO, redox) may be affected due to aeration or agitation (Kaminski 2003). Quarterly well samples were collected using a low-flow sampling method. Physical parameters were measured as well as inorganic and organic components: volatile organic compounds (VOCs), total and dissolved iron, total and dissolved ferrous iron, methane, ethane, ethene, CaCO3 alkalinity, and total organic carbon. Select duplicate samples were taken for quality assurance and for matrix spike samples, as were trip blanks. Analyses were conducted by BMT or their contract commercial laboratory using standard approved EPA methods (US EPA 1996, 1998; BMT 2014).

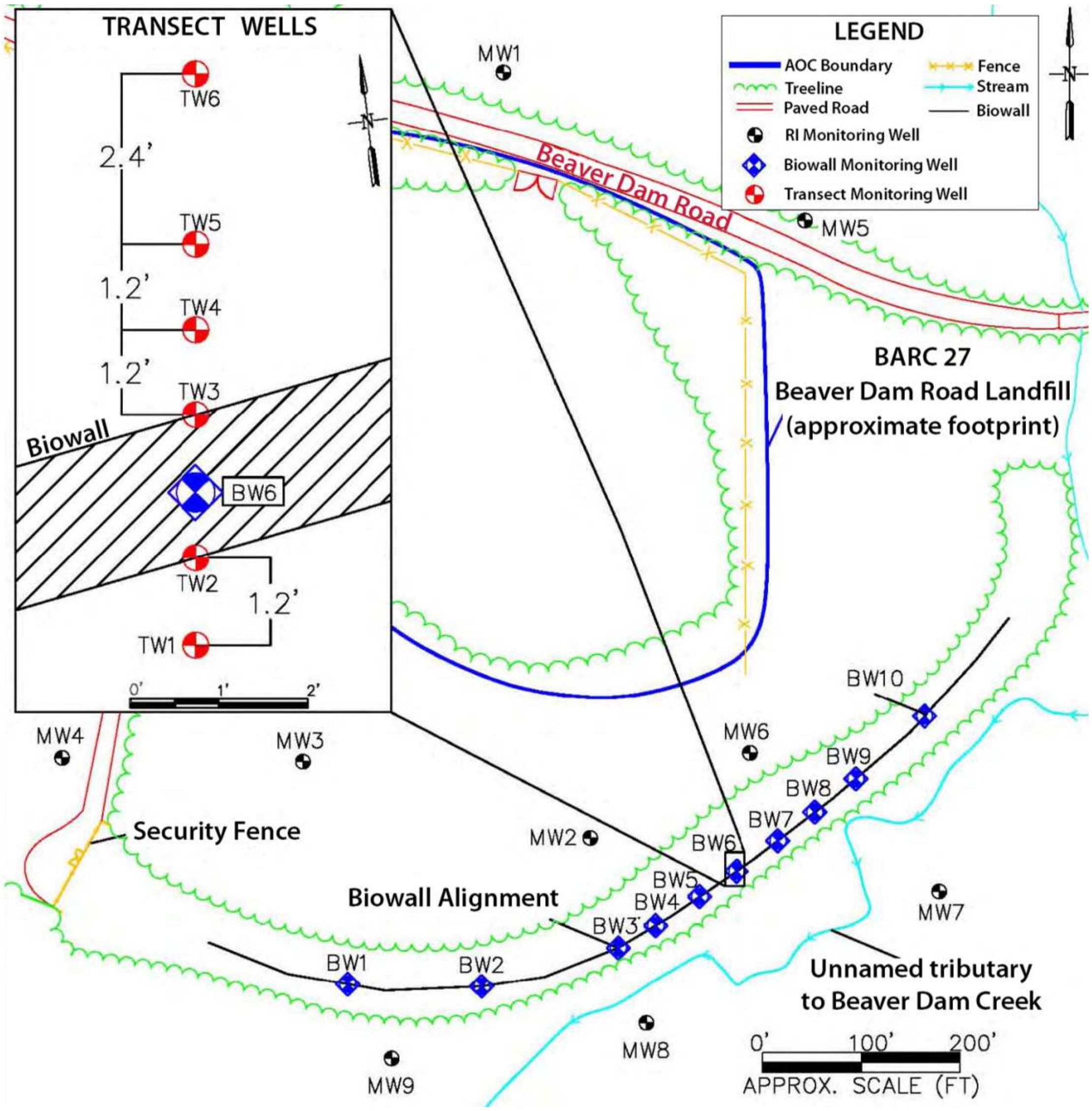

Figure 1-1: Beaver Dam Road landfill site map with monitoring well locations. Transect wells (TW), biowall wells (BW), and remedial investigation wells (MW) depicted.

<u>Results and discussion</u>

TCE fate in the reactors

VOC concentrations were measured in the headspace of each reactor, and a single-phase decay model was used to calculate the TCE dissipation rate constant, k_{TCE} (Table 2). A decrease in TCE concentration was detected in the headspace of all the reactors including the controls (Fig. 2, Online Resource 2), but differences were observed in the calculated TCE dissipation rate constants between the three groups. The largest variation between the three groups was in the unamended M4C3 reactors (G0 Fe0) where k_{TCE} ranged from 8.8×10^{-6} to 6.6×10^{-3} day−1. For all the other reactor types, k_{TCE} varied by no more than a factor of 3 among the three groups. These data indicate that despite efforts to homogenize the materials before packing the reactors, unique microcosm effects were present and could not be eliminated. Therefore, quantitative comparisons were typically made within a group, and qualitative trends were noted between groups.

Table 1-2: Trichloroethylene (TCE) dissipation rates calculated

Group 1	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**	**M4C3 G0 Fe10**	**M4C3 G10 Fe10**	**M4C3 G30 Fe10**	**M1C1 G0 Fe0**	**M1C1 G10 Fe10**	**M1C1 G0 Fe10**	**Sand Control**	**Soil Control**
k_{TCE} (d^{-1})	6.58E-03	7.01E-02	6.07E-02	4.40E-02	8.35E-02	2.58E-02	3.59E-02	2.77E-02	3.45E-02	3.81E-02
R^2	0.88	0.93	0.98	0.98	0.91	0.93	0.97	0.97	0.77	0.71
N	19	19	29	52	33	59	36	42	60	54

Group 2	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**	**M4C3 G0 Fe10**	**M4C3 G10 Fe10**	**M4C3 G30 Fe10**	**M1C1 G0 Fe0**	**M1C1 G10 Fe10**	**M1C1 G0 Fe10**	**Sand Control**	**Soil Control**
k_{TCE} (d^{-1})	1.15E-05	3.58E-02	3.05E-02	4.45E-02	3.91E-02	3.0E-02	1.31E-02	1.99E-02	1.13E-02	5.24E-03
R^2	0.85	0.99	0.97	0.98	0.99	0.95	0.87	0.98	0.78	0.64
N	9	17	32	30	30	52	43	44	56	51

Group 3	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**	**M4C3 G0 Fe10**	**M4C3 G10 Fe10**	**M4C3 G30 Fe10**	**M1C1 G0 Fe0**	**M1C1 G10 Fe10**	**M1C1 G0 Fe10**	**Sand Control**	**Soil Control**
k_{TCE} (d^{-1})	8.80E-06	3.06E-05	4.21E-02	2.35E-02	4.81E-02	3.58E-02	1.85E-02	2.10E-02	3.07E-02	3.01E-02
R^2	0.96	0.85	0.97	0.95	0.97	0.97	0.95	0.97	0.84	0.81
N	17	21	42	28	39	51	43	51	45	50

The relatively rapid decrease of TCE in the headspace in nearly all the reactors with the M4C3 mixture indicates that this base has a strong affinity for TCE sequestration (Fig. 2 and Online Resource 2). Most likely, the larger mulch content of the M4C3 bulk mixture increased the TCE sorption/degradation capacity of the support material (Ahmad et al. 2007). By day 40, a reduction in TCE headspace concentration of at least 70% was observed for the amended M4C3 reactors and a 55% reduction in the amended M1C1 reactors, whereas a reduction of 47 and 53% was observed in the unamended M4C3 and M1C1 reactors, respectively. After day 67, no detectable TCE in the headspace of the unamended M4C3 reactors was seen, but the unamended M1C1 reactors contained 7 to 35% of the starting TCE headspace concentration. The sand and soil controls contained approximately 60 and 36%, respectively, of the initial TCE headspace concentration at the conclusion of the experiment.

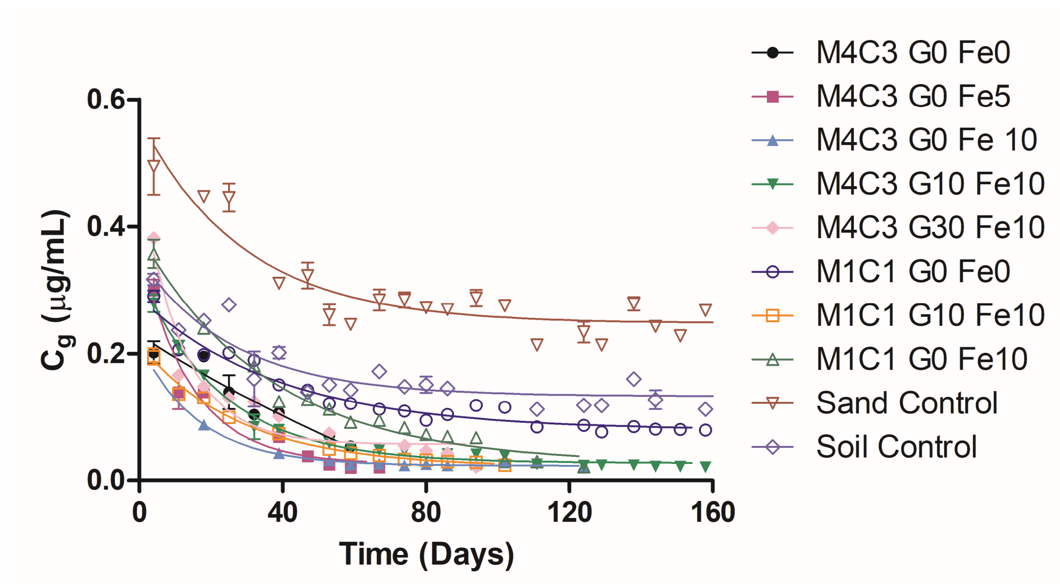

Figure 1-2: Single-phase decay of trichloroethylene measured in headspace of group 1 batch reactors with either M4C3 or M1C1 mulch/compost fill material ratios. Glycerol (G) added at 0, 10, or 30 mL/L fill material. Zero-valent iron (Fe) added at 0, 5, or 10 mL/L fill material.

The fastest k_{TCE} (5.7×10^{-2} day^{-1} $\pm$ 2.3×10^{-2} day^{-1}) was observed in the M4C3 batch reactor containing high glycerol and ZVI (G30 Fe10), whereas the slowest k_{TCE} (2.2×10^{-3} day^{-1}; SD = 3.8×10^{-3} day^{-1}), not including the controls, was for the unamended M4C3 reactor. Incorporating ZVI into the bulk material increased kTCE at least threefold. Adding glycerol to the bulk material that contained ZVI did not demonstrate a marked effect on TCE dissipation rate except at the highest dose (G30). The TCE dissipation rates observed in this study fall in the range of the first-order rate constants and decay coefficients observed elsewhere (Table 3). Please observe that negative values represent first-order rate constants and positive values represent decay coefficients.

Table 1-3: First-order rate constants and decay coefficients of TCE and its degradation products from literature

Reaction or Parameter	Estimated first-order rate constant (a), or decay coefficient (b) (d^{-1})	Source	Notes
TCE → DCE	-1.2×10^{-5} to -6.7×10^{-5} (a)	Davis et al. 2002	Natural attenuation; 15°C
TCE	1.4×10^{-4} to 2.5×10^{-3} (b)	*modified* Wiedemeier et al. 1996	Natural attenuation; groundwater temperature (GW)
	0.224, 1.2 (b)	Ozturk et al. 2012	Eucalyptus mulch, compost; room temperature (RT)
	0.003 - 0.37 (b)	Schaerlaekens et al. 1999; Mulligan and Young 2004	Natural attenuation; GW
	0.230 (b)	Henry et al. 2003; Ahmad et al. 2007	Biowall
	0.114 (b)	GSI 2001; Ahmad et al. 2007	Biowall
	0.185 (b)	GSI 2004; Ahmad et al. 2007	Biowall
DCE → VC	-1.4×10^{-6} to -6.2×10^{-6} (a)	Davis et al. 2002	Natural attenuation; 15°C
trans-1,2-DCE	0.026 (b)	Ozturk et al. 2012	Compost
	0.007 - 0.017 (b)	Kassenga et al. 2004; Schaerlaekens et al. 1999	Upflow treatment wetland system; pump-and-treat system
cis-1,2-DCE	0.0254, 0.064 (b)	Ozturk et al. 2012	Eucalyptus mulch, compost
	0.002 - 1.57 (b)	Schaerlaekens et al. 1999; Kassenga et al. 2004	Pump-and-treat; upflow treatment wetland system
VC → ETH	-3.7×10^{-3} to -1.1×10^{-2} (a)	Davis et al. 2002	Natural attenuation; 15°C
	3.3×10^{-4} to 7.1×10^{-3} (b)	*modified* Wiedemeier et al. 1996	Natural attenuation

A one-way ANOVA comparing the rate constants for the treatments and the controls was conducted within each group. No significant differences in the TCE dissipation rates were observed among all the reactors containing compost and mulch, but the TCE dissipation rates in all the compost and mulch reactors were significantly lower than the rates observed in the control reactors of sand and soil. These data indicate that despite the lack of significant separation between treatments, any combination of compost and fill materials selected for field application would be preferable to natural attenuation.

DCE dissipation

No analytical distinction was made between *cis*- and *trans*-DCE over the course of this study. The DCE produced was presumed to be predominantly the *cis*-DCE form, prevalent during reductive dechlorination as opposed to the trans-form which is rarely detected under these conditions (Vogel and McCarty 1987). In all three groups, DCE was detected in only two of the ten reactor conditions, M4C3 with no glycerol and no or low ZVI (G0 Fe0 and G0 Fe5) (Fig. 3 and Online Resource 3). The amount of DCE produced in all these reactors was below the MCL of 70 ppb. In addition, no significant differences were observed between groups regarding the DCE dissipation rate (Table 4). Overall, DCE decreased at an average rate of 1.5×10^{-4} day^{-1} (SD = 4×10^{-5} day^{-1}).

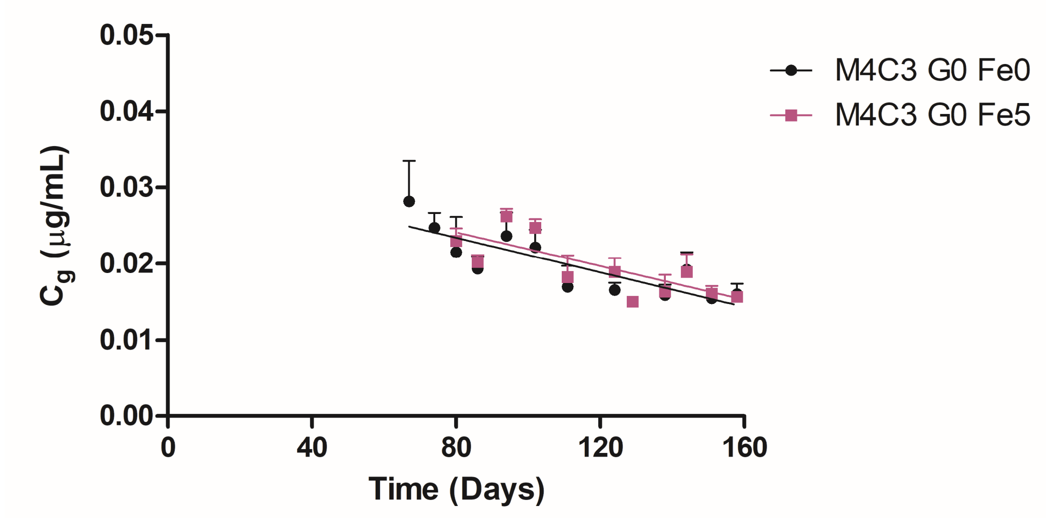

Figure 1-3: *cis-/trans*-1,2-Dichloroethylene measured in headspace of group 1 batch reactors with either M4C3 or M1C1 mulch/compost fill material ratios. Glycerol (G) added at 0, 10, or 30 mL/L fill material. Zero-valent iron (Fe) added at 0, 5, or 10 mL/L fill material.

Table 1-4: Dichloroethylene (DCE) dissipation rate.

	Group 1		Group 2		Group 3	
	M4C3 G0 Fe0	**M4C3 G0 Fe5**	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**
k_{DCE} (d^{-1})	1.13E-04	1.10E-04	1.56E-04	2.02E-04	1.14E-04	1.79E-04
R^2	0.55	0.56	0.51	0.42	0.78	0.82
N	33	29	46	11	34	29

VC production and degradation

VC was observed in all the reactors except the soil control in group 2 and the unamended M4C3 and M1C1 reactors (G0 Fe0). The earliest measurable VC concentrations were on day 11 in the three reactor types containing glycerol (G10 and G30), whereas measurable VC was not observed until after day 111 for those reactors containing only ZVI (G0 Fe5 and G0 Fe10) (Fig. 4 and Online Resource 4). This is consistent with a recent study where glycerol was injected into an aquifer contaminated with *cis*-DCE giving rise to dechlorination with a concomitant accumulation of VC (Atashgahi et al. 2017). The amount of VC produced in all the reactors was above the MCL of 2 ppb. The large concentration of VC in the headspace could be due to the slowed conversion of VC to ethylene as these types of reducing conditions have been shown to dictate the rate-limiting step of complete dechlorination. In previous studies, the most common impedance was the conversion of VC to ethylene under methanogenic conditions (Freedman and Gossett 1989; Davis et al. 2002; Tandoi et al. 1994).

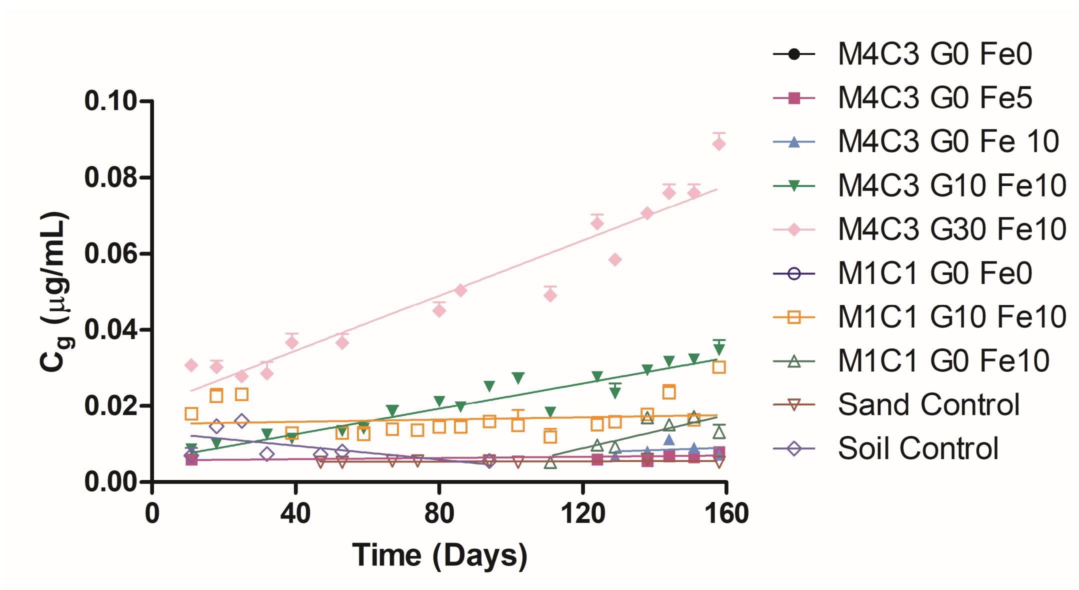

Figure 1-4: Vinyl chloride measured in headspace of group 1 batch reactors with either M4C3 or M1C1 mulch/compost fill material ratios. Glycerol (G) added at 0, 10, or 30 mL/L fill material. Zero-valent iron (Fe) added at 0, 5, or 10 mL/L fill material.

The VC production rate among the three groups was fairly consistent (Table 5). The fastest apparent production of VC, k_{VC}=5.3×10^{-4} day^{-1} ± 2.1×10^{-4} day^{-1}, was M4C3 with high glycerol and ZVI (G30 Fe10), while the slowest, k_{VC}=2.9×10^{-5} day^{-1} ± 2.5×10^{-5} day^{-1} was M4C3 with ZVI and no glycerol (G0 Fe5 and G0 Fe10). This difference was significant in all three groups ($P < 0.05$). In groups 2 and 3, the M1C1 low glycerol and high ZVI (G10 Fe10) reactors were also significantly different ($P < 0.05$) when compared to the M4C3 no and low glycerol with high ZVI (G0 Fe10 and G10 Fe10) reactors and the M4C3 no glycerol and low ZVI reactors (G0 Fe5). All reactors containing any amount of glycerol saw a gradual increase in the amount of VC produced. Note that these production values are slower than the decay rates reported in Table 3. Based on this rate difference, over time the system should be able to trap and degrade this compound faster than it is being produced.

Table 1-1: Vinyl chloride (VC) production rate.

Group 1	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**	**M4C3 G0 Fe10**	**M4C3 G10 Fe10**	**M4C3 G30 Fe10**	**M1C1 G0 Fe0**	**M1C1 G10 Fe10**	**M1C1 G0 Fe10**	**Sand Control**	**Soil Control**
k_{VC} (d^{-1})	-	7.88E-06	3.15E-05	1.68E-04	3.62E-04	-	1.46E-05	2.17E-04	1.93E-06	9.05E-05
R^2	-	0.21	0.04	0.88	0.92	-	0.02	0.58	0.07	0.26
N	-	13	6	44	40	-	46	16	8	15

Group 2	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**	**M4C3 G0 Fe10**	**M4C3 G10 Fe10**	**M4C3 G30 Fe10**	**M1C1 G0 Fe0**	**M1C1 G10 Fe10**	**M1C1 G0 Fe10**	**Sand Control**	**Soil Control**
k_{VC} (d^{-1})	-	6.73E-05	5.02E-05	3.08E-04	7.59E-04	-	2.20E-04	1.52E-04	6.15E-05	-
R^2	-	0.24	0.44	0.81	0.95	-	0.80	0.52	0.40	-
N	-	23	27	54	53	-	49	17	9	-

Group 3	**M4C3 G0 Fe0**	**M4C3 G0 Fe5**	**M4C3 G0 Fe10**	**M4C3 G10 Fe10**	**M4C3 G30 Fe10**	**M1C1 G0 Fe0**	**M1C1 G10 Fe10**	**M1C1 G0 Fe10**	**Sand Control**	**Soil Control**
k_{VC} (d^{-1})	-	1.21E-05	5.65E-06	2.04E-04	4.58E-04	-	3.23E-04	2.00E-04	9.75E-05	2.37E-04
R^2	-	0.24	0.01	0.65	0.87	-	0.73	0.93	0.51	0.93
N	-	14	31	32	40	-	38	33	31	15

Because an increase in ZVI dose did not necessarily correspond to a consistent change in VC production rate, the relationship between the concentration of ZVI and k_{VC} is less clear. The measurable amounts of DCE and VC were lower in the reactors containing high ZVI (Fe10) versus low ZVI (Fe5). Thus, 1% v/v ZVI addition may be sufficient to promote long-term reduction when coupled with the iron deposits naturally found at the site.

Acetylene and ethylene production

The rate of production of acetylene and ethylene was two to four orders of magnitude greater than the production rate of VC. An exponential growth model was used to describe the generation of ethylene and acetylene (data not shown). Three mixtures, M4C3 high glycerol and ZVI (G30 Fe10), and M1C1 with no glycerol or low ZVI (G0 Fe0 and G0 Fe5) produced the least amount of acetylene and ethylene, less than 1% of the final concentration of the other combinations. The M1C1 mixture with low glycerol and high ZVI (G10 Fe10) consistently produced the most acetylene and ethylene. The addition of ZVI had an overall positive impact on the reductive dechlorination of TCE; however, when used in combination with the high dose of glycerol (G30), it did not promote the complete dechlorination of TCE.

pH, redox conditions, and total carbon content in the reactors

Although no action was taken to influence pH in the batch reactors, the measured pH values indicated a habitable environment for microbial colonization (pH 6.3–7.5). The pH of the soil control at the conclusion was slightly more acidic (pH 5–6), while that of the sand control was much more basic (pH 10.3–10.9) (Online Resource 5). The redox condition (ORP) measured in the two reactor groups tested demonstrated reducing conditions (Online Resource 6). The average ORP of the unamended M4C3 and M1C1 reactors was −184.0 mV (SD = ± 20.1 mV) and − 242.0 mV (SD = ±58.2 mV), respectively. The reducing conditions of the ZVI only reactors increased in strength as the dose increased, and the inclusion of glycerol with ZVI further increased the strength of the reducing conditions as dose increased. The sand and soil controls did not demonstrate consistent behavior as reducing conditions were observed in the group 1 controls, while oxidizing conditions were found in the group 2 controls.

The total carbon content measured at the conclusion of the experiment was not consistent between groups though reactors containing glycerol had a higher total carbon percentage than the unamended reactors. The organic carbon content (f_{OC}) calculated from the Shimadzu SSM-5000 carbon measurements showed greater consistency between groups, though the values did not follow a clear trend with respect to amendment (Online Resource 7). The f_{OC} for the unamended M4C3 reactors ranged from 0.05 to 0.10% and a had a calculated K_d of 2.15 L/kg. Reactors with larger f_{OC} values had smaller TCE concentrations in the headspace.

VOC measurements in the biowall

Although TCE was detected in all of the transect wells, it was only found in five of the ten biowall wells and in two remedial investigation wells (Online Resource 8a, b). The level of TCE measured within
the center of the biowall (BW6) decreased from 9 to 0.7 ppb with the exception of two brief spikes in March 2015 and April 2016. The TCE concentrations in transect wells from TW06 to TW04 were also relatively large in March 2015. These concentration spikes do not correspond with any fluctuations in depth-to-water table measurements; precipitation data 1 month prior to each spike event do not show heavy rain or snow fall which would raise the water level and possibly promote an

influx of TCE. The slow groundwater flow rate and the effective biowall thickness of approximately 0.9 m (BMT Designers and Planners 2016) suggest 26 days are necessary for the current slug of upstream groundwater to make its way through the structure before being monitored at TW02.

The largest concentrations of both cis-1,2-DCE and VC within the biowall were at BW06 (Online Resources 9 and 10), though moving across the structure, the highest concentration of these compounds was at the lagging edge (TW02). BW06 also showed an upward trend in ethylene concentration
(Online Resource 11). Though low, this trend indicates that the biowall fill materials are performing as expected and that the materials are biologically active as ethylene generation is indicative of active reductive dechlorination. The DCE and VC downstream concentrations were greater than their incoming concentrations with a moderate oscillation in the production of DCE. The production of VC was very rapid between March 2014 and September 2014: the concentrations increased 4–6-fold at TW01 through TW04 and BW06. After September 2014, the generation of VC steadily decreased with the upstream concentration appearing to converge at approximately 8 ppb and the downstream concentration at 19 ppb.
Since VC is the most toxic degradation product generated by the reductive dechlorination process, the concentrations measured downstream of the structure must be below the MDL in order for the biowall to be completely effective.

Physical and geochemical properties of the biowall

The average groundwater temperature was 13 ± 2 °C. Total iron and dissolved iron (Online Resource 12) were measured at each biowall well and did not exhibit any overall trend. The pH within the biowall remained constant (pH ~ 6) until December 2014 (Online Resource 13a). After this date, the pH fluctuated between pH 4 and 8. Along the transect line, the pH was relatively stable at a value of 6 (with the exception of TW05 and TW06) until December 2014, when, again, the pH fluctuated between 4 and 8 (Online Resource 13b). It may become necessary to intervene in order to maintain a favorable pH range for the microbial community; however, the manner of this intervention requires further study both for type and feasibility.

The redox condition (ORP) measured at the biowall wells showed seasonal variation with respect to oxidizing or reducing conditions while the ORP measured in the transect wells was more stable and kept either oxidizing or reducing conditions. Upstream of the biowall at TW06 and TW05 were consistently under oxidizing conditions, while TW03 through TW01 were consistently reduced, though as of January 2016 these readings began trending toward becoming more oxidized (Online Resource 14a, b). Unlike the other upstream locations, the ORP measured at TW04 oscillated between reducing to (slightly) oxidizing conditions. It is unclear why the ORP at TW04 was not more similar to the other upstream sampling sites. This gradual shift of ORP toward more oxidizing conditions may partially explain the decrease in VC concentration.

TOC concentrations within the biowall dropped more dramatically in some areas compared to others, possibly indicating an uneven aging of the material due to a combination of natural degradation and microbial activity (Online Resource 15). The BW06 site demonstrates a more steady release of TOC at about 100 mg/L over the course of a little more than a year, while BW09 released only about 25 mg/L. The rapid release of TOC may increase subsidence and may present a hazard to wildlife as well as change overland flow patterns. Rapidly aging portions of the biowall will need structural attention sooner than other areas and may include the addition of "sacrificial" carbohydrate-containing compounds to divert the attention of the microbial community's metabolism away from the fill materials. The $CaCO_3$ measured within the biowall has a very broad range and no discerning pattern regarding wall position. However, there is a slight global downward trend over the course of the monitoring period. This may have contributed to the variability in pH.

Hazard quotient changes

Despite the elevated level of VC downstream of the biowall, a preliminary screening level risk assessment shows an 88% decrease in relative toxicity downstream of the biowall from upstream. This was determined by initially calculating a hazard quotient (HQ) for each compound at each position; the observed field concentration, or exposure point concentration, was divided by the corresponding regional screening level (RSL), in this case the relevant MCL (US EPA 2016b). The upstream values were then added together as are the downstream values, and the difference between these numbers indicates the relative difference in toxicity between the two positions.

Although this HQ calculation is limited in that ecological risk, exposure pathways, duration, carcinogenicity, and contaminant contact method are not considered (US EPA 2016c), this calculation is helpful in assessing any changes in the contamination risk.

Conclusions

The unamended M4C3 mulch/compost combination was selected as the biowall fill material during the course of the batch reactor experiments. In the laboratory, the unamended M4C3 mixture demonstrated rapid sorption and degradation of TCE without buildup of the toxic DCE and some generation of VC.

In the field, the TCE concentrations measured downstream of the biowall were in general between 2 and 10% of the upstream concentrations indicating sorption and some degradation, as evidenced by the increase in ethylene concentration from June 2014 to September 2016. TCE was not detected in TW02 and TW01 from June 2016 to September 2016. The reducing conditions inside the biowall have supported the production of cis-1,2-DCE and VC, and at present, the VC concentration downstream of the biowall is almost twice as high as that measured in the center of the structure (BW06). Though the relative overall toxicity downstream of the biowall is 88% lower than the toxicity upstream (as of 2016), promoting the conversion of VC into ethylene or some other nontoxic byproduct is a high priority and currently being studied.

Further investigation of glycerol or another easily accessible carbohydrate-containing molecule would be useful since the microbial metabolism of the bioavailable organic carbon fraction is occurring more rapidly than the degradation of the fill material. This indication of high microbial activity suggests that another carbon source will be necessary to keep up with demand at some point in the future. Data from this study showed that addition of glycerol to the reactors did not have an adverse effect on the TCE degradation, though the amount of VC produced was noticeably higher. The addition of ZVI to the reactors provided sufficient reducing conditions to degrade TCE and its degradation products. The amount of VC measured in the headspace of the ZVI-only reactors contained levels five to ten times lower than those containing some measure of glycerol, though slightly above the MCL. The antimicrobial aspect of ZVI is of concern as a biologically active biowall is desired to improve degradation capability of the engineered structure. New studies have been initiated to explore the toxicity of ZVI at the doses studied here, and to determine if native soil bacteria and other TCE-degrading cultures can survive in the biowall material containing ZVI. By improving the living conditions within the biowall for select microbial communities, a corresponding increase in dechlorination rates should be evident.

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manuscript describes original work and the authors do not have any conflicts of interest.

Compliance with ethical standards

Disclaimer

Mention of specific products is for identification purposes only and does not imply endorsement by the US Department of Agriculture nor the Federal Government to the exclusion of other suitable products or suppliers.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary material/online resources

Figure 1-5 (Online Resource 1): Chromatogram and retention times of trichloroethylene and select degradation products

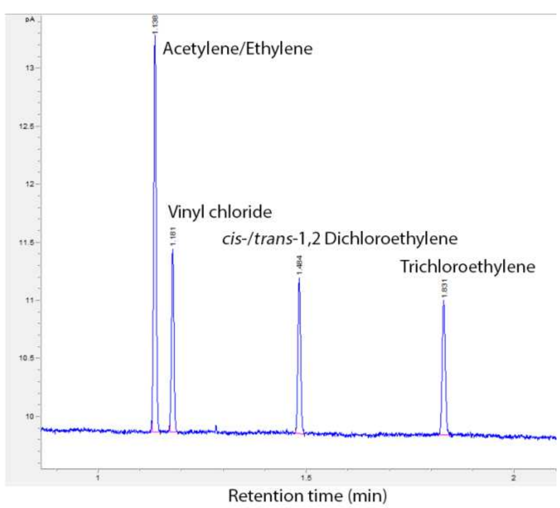


Figure 1-6 (Online Resource 2): Single-phase decay of trichloroethylene measured in headspace of batch reactors with either M4C3 or M1C1 mulch/compost fill-material ratios. Glycerol (G) added at 0, 10, or 30 mL/L fill-material. Zero valent iron (Fe) added at 0, 5, or 10 mL/L fill-material. (a) Group 2; (b) Group 3

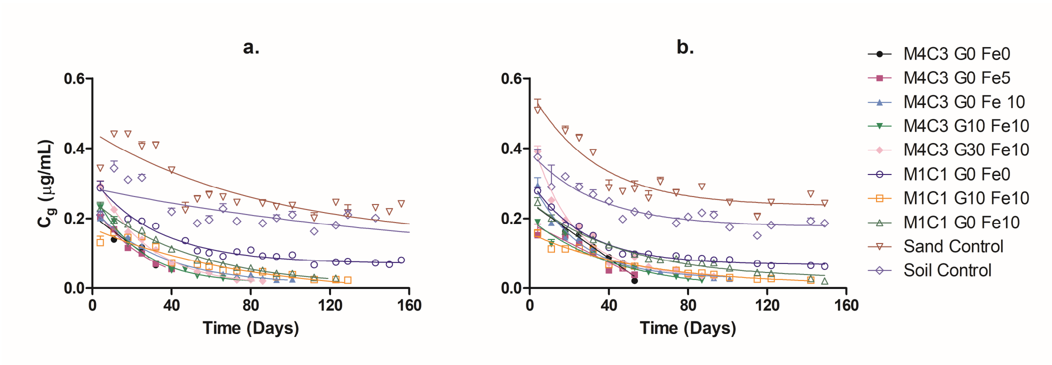


Figure 1-7 (Online Resource 3): *cis-/trans*-1,2 dichloroethylene measured in the headspace of batch reactors with either M4C3 or M1C1 mulch/compost fill-material ratios. Glycerol (G) added at 0, 10, or 30 mL/L fill-material. Zero valent iron (Fe) added at 0, 5, or 10 mL/L fill-material. (a) Group 2; (b) Group 3

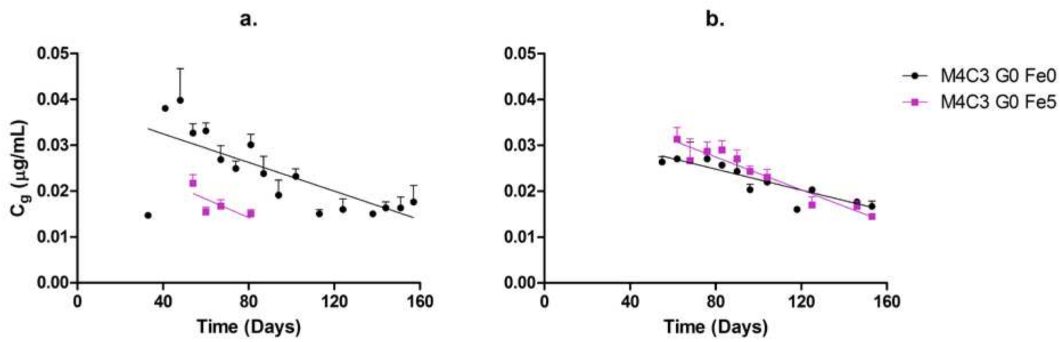


Figure 1-8 (Online Resource 4): Vinyl chloride measured in the headspace of batch reactors with either M4C3 or M1C1 mulch/compost fill-material ratios. Glycerol (G) added at 0, 10, or 30 mL/L fill-material. Zero valent iron (Fe) added at 0, 5, or 10 mL/L fill-material. (a) Group 2; (b) Group 3

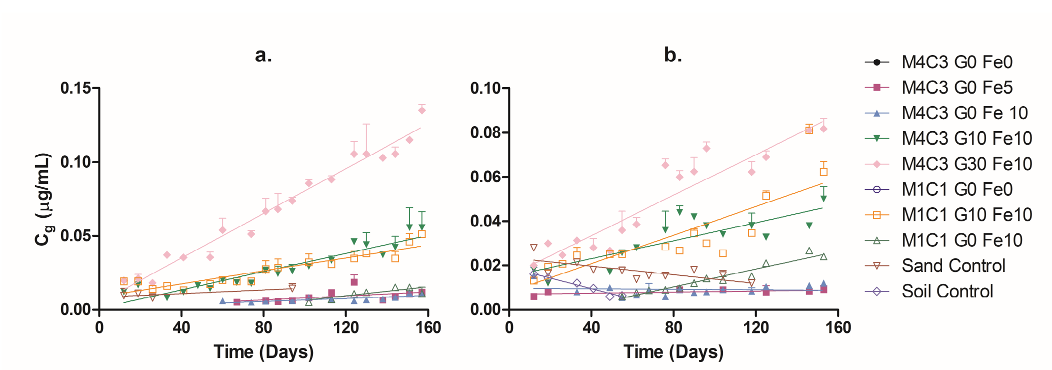


Table 1-6 (Online Resource 5): Average pH values measured of batch reactor materials

Reactor	Group 1		Group 2	
	Mean	s.d	Mean	s.d.
M4C3: G0 Fe0	6.5	-	6.5	0.09
M4C3: G0 Fe5	6.8	0.03	7.0	0.03
M4C3: G0 Fe10	6.9	-	7.2	0.02
M4C3: G10 Fe10	NV	-	7.0	0.03
M4C3: G30 Fe10	6.3	0.03	NV	-
M1C1: G0 Fe0	6.6	0.03	6.8	0.11
M1C1: G10 Fe10	7.1	0.02	7.2	0.07
M1C1: G0 Fe10	7.3	0.04	7.5	0.02
Sand Control	10.3	0.62	10.9	0.04
Soil Control	6.0	0.01	5.0	0.03

NV = No Value; reactor broke and no value recorded

Table 1-7 (Online Resource 6): Oxidation reduction potential measured in batch reactors

Reactor	Group 1			Group 2		
	Eh (mV)	ORP (mV)	s.d	Eh (mV)	ORP (mV)	s.d.
M4C3: G0 Fe0	32.3	-169.8	-	3.9	-198.2	8.9
M4C3: G0 Fe5	-33.0	-235.1	3.6	-38.7	-240.8	10.0
M4C3: G0 Fe10	-74.9	-277.0	-	-31.4	-233.5	29.7
M4C3: G10 Fe10	NV	NV	-	-51.3	-253.4	9.5
M4C3: G30 Fe10	-160.5	-362.6	6.9	NV	NV	-
M1C1: G0 Fe0	-81.1	-283.2	20.1	1.3	-200.8	19.2
M1C1: G10 Fe10	-15.9	-218.0	8.1	-245.1	-447.1	202.3
M1C1: G0 Fe10	-122.6	-324.7	194.1	-123.2	-325.3	18.7
Sand Control	119.9	-82.2	12.2	223.3	21.2	36.9
Soil Control	32.7	-169.4	6.5	322.4	120.3	6.2

NV = No Value; reactor broke and no value recorded

Table 1-8 (Online Resource 7): Average carbon content of each reactor post-experiment

Reactor	Group 1		Group 2	
	%TC (± SD)	*foc* (%)	%TC (± SD)	*foc* (%)
M4C3: G0 Fe0	5.0 (± 0.9)	0.05	10.4 (± 1.7)	0.10
M4C3: G0 Fe5	8.3 (± 1.3)	0.08	9.6 (± 3.4)	0.11
M4C3: G0 Fe10	12.1 (± 2.5)	0.11	7.2 (± 0.6)	0.07
M4C3: G10 Fe10	NV	NV	13.4 (± 10.3)	0.07
M4C3: G30 Fe10	9.1 (± 3.2)	0.07	NV	NV
M1C1: G0 Fe0	2.3 (± 0.4)	0.02	3.9 (± 0.4)	0.04
M1C1: G10 Fe10	4.6 (± 0.5)	0.04	4.3 (± 0.7)	0.04
M1C1: G0 Fe10	2.2 (± 0.2)	0.02	3.6 (± 0.7)	0.04
Sand Control	0.1	0.001	0.1	0.001
Soil Control	0.4	0.004	0.3 (± 0.01)	0.003

NV = No Value; reactor broke and no value recorded

Figure 1-9 (Online Resource 8): Trichloroethylene measured in groundwater at study site. (a) Biowall (BW) and Remedial Investigation (MW) wells; (b) Transect (TW) wells

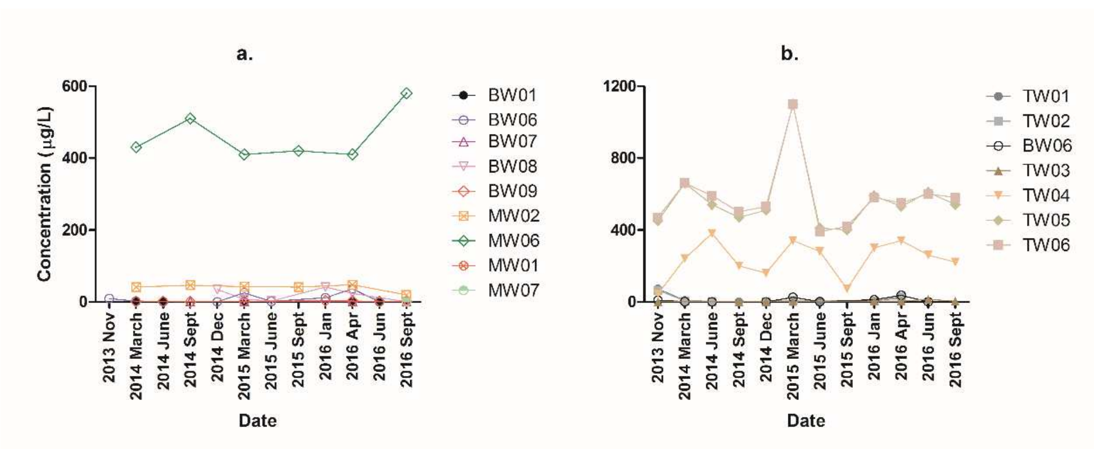


Figure 1-10 (Online Resource 9): *cis*-1,2-Dichloroethylene measured in groundwater at study site. (a) Biowall (BW) and Remedial Investigation (MW) wells; (b) Transect (TW) wells

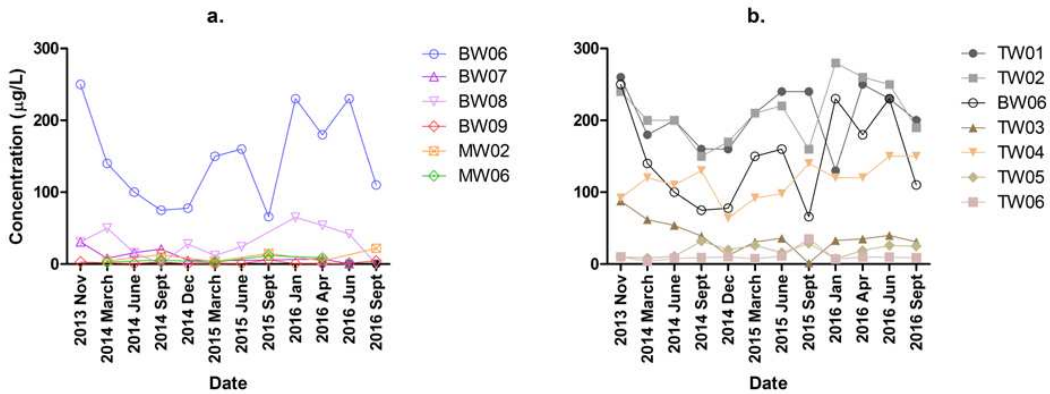


Figure 1-11 (Online Resource 10): Vinyl chloride measured in groundwater at study site. (a) Biowall (BW) and Remedial Investigation (MW) wells; (b) Transect (TW) wells

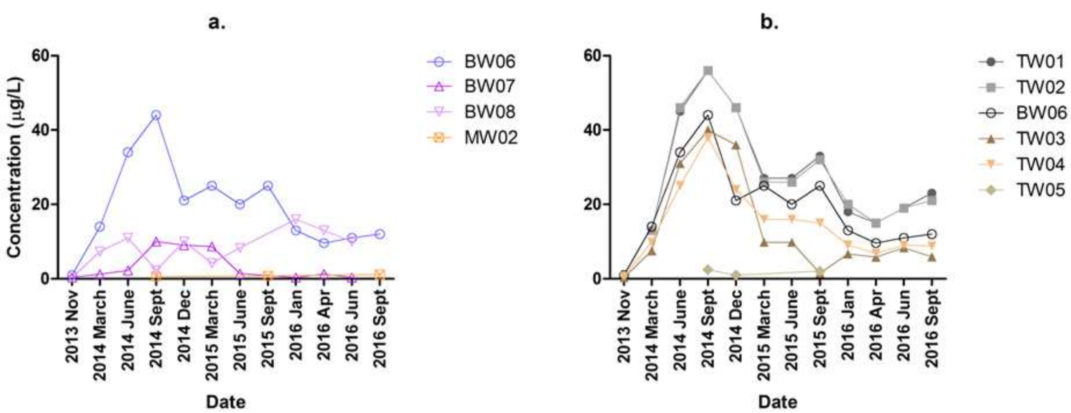


Figure 1-12 (Online Resource 11): Ethylene measured in groundwater at study site from the Biowall (BW), Remedial Investigation (MW), and Transect (TW) wells

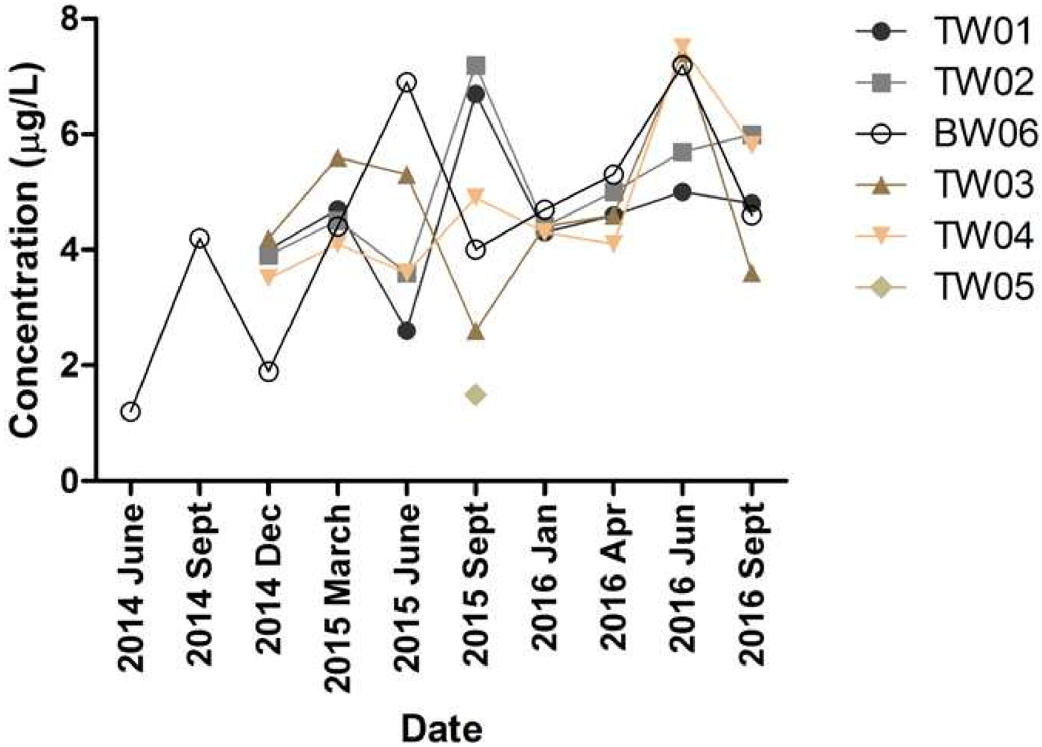


Figure 1-13 (Online Resource 12): Iron measured in groundwater at study site. (a) Total iron, unfiltered; (b) Dissolved iron, filtered

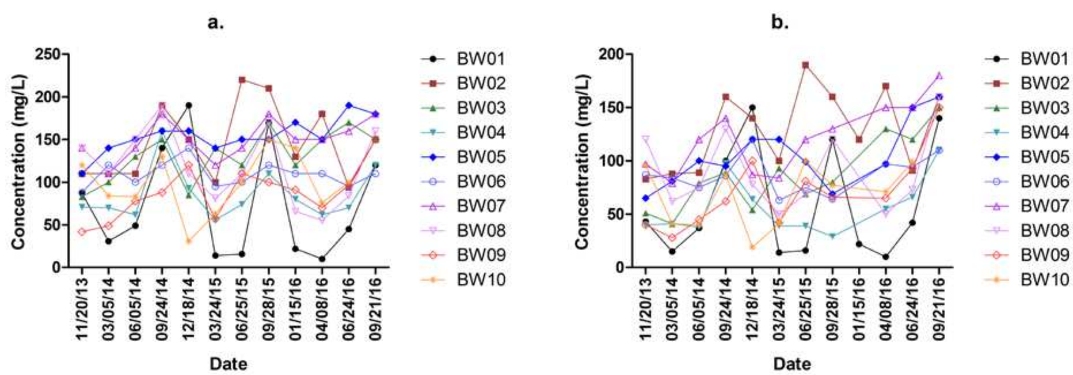


Figure 1-14 (Online Resource 13): pH measured in groundwater at study site. (a) Biowall (BW) wells; (b) Transect (TW) wells

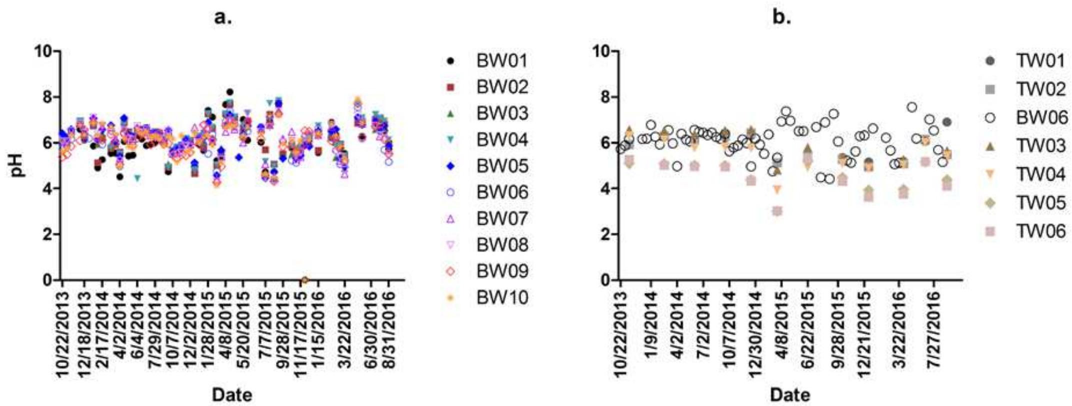


Figure 1-15 (Online Resource 14): Oxidation/reduction potential measured in groundwater at study site. (a) ORP at BW sites; (b) ORP at TW sites; (c) Eh at BW sites

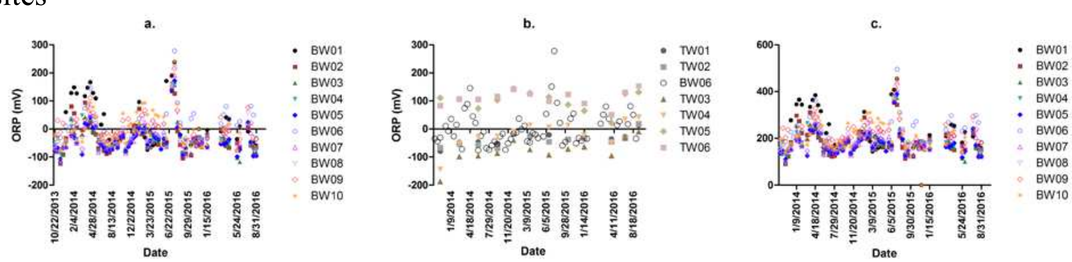
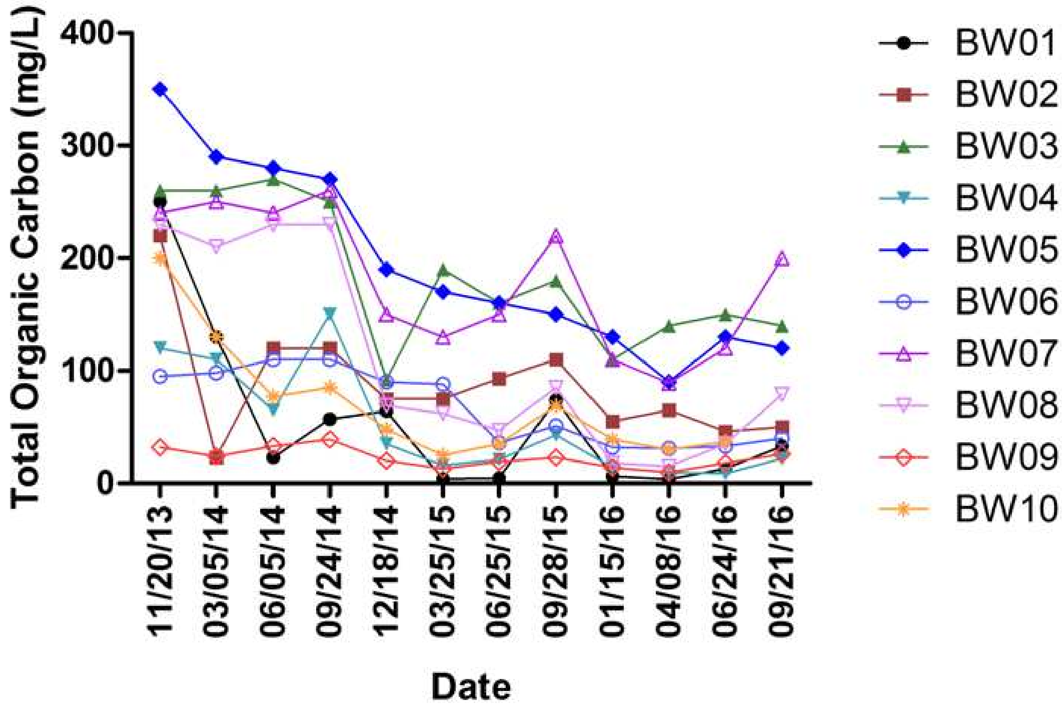


Figure 1-16 (Online Resource 15): Total organic carbon (TOC) measured in groundwater at study site Biowall (BW) wells



Epilogue

Follow-up experiment

At the conclusion of the batch reactor experiment outlined in the manuscript of this chapter, one test group was set aside to investigate the properties of the "spent" material and how this would affect their disposal according to the EPA standard methods (FR 1998A,B; CFR 2012). As the biowall ages, its fill-material will have to be replaced, which may include the partial excavation and/or removal of the spent fill-material. The disposal protocol for this type of material depends on the contaminants (if they can be found on the US EPA's "Lists of Hazardous Wastes"), their concentrations, and type of material being disposed (FR 1998A,B). As such, the maximum allowable total concentration of TCE, 1,1-DCE, trans-1,2-DCE, and VC are 6.08×10^{0}, 1.40×10^{-1}, 9.14×10^{1}, 4.68×10^{-2} mg/kg, respectively (CFR 2012). The maximum allowable leachate concentration is more restrictive with a different set of rules; this concentration is determined by following the method outlined in the Toxicity Characteristic Leaching Procedure (test method 1311) (US EPA 1992). The batch reactors were briefly opened, exposing the contents to an aerobic environment then tightly sealed again in order to investigate if the fill-material would release any previously trapped/degraded TCE, DCE, and VC. While headspace samples were collected 24 and 48 hours after this aerobic exposure, the experiment could not continue due to the growth of mold in the reactors. A repetition of this experiment would be beneficial in order to understand how tightly bound the volatile contaminants are to the fill-material, and to develop a treatment method for the safe, non-hazardous disposal of any spent material

References

Air Force Center for Engineering and the Environment (AFCEE) (2008) Technical protocol for enhanced anaerobic bioremediation using permeable mulch biowalls and bioreactors. AFCEE Technical Directorate. May 2008

Ahmad F, McGuire TM, Lee RS, Becvar E (2007) Considerations for the design of organic mulch permeable reactive barriers. Remediation 18(1):59-72

Arnold WA, Roberts AL (2000) Pathways and kinetics of chlorinated ethylene and chlorinated acetylene reaction with Fe(0) particles. Environ Sci Technol 34:1794-1805

Atashgahi S, Lu Y, Zheng Y, Saccenti E, Suarez-Diez M, Ramiro-Garcia J, Eisenmann H, Elsner M, Stams AJM, Springael D, Dejonghe W, Smidt H (2016) Geochemical and microbial community determinants of reductive dechlorination at a site biostimulated with glycerol. Environ Microbiol. doi:10.1111/1462-2920.13531

BMT Entech Inc. (2008) *BARC Fact Sheet: Beaver Dam Road Landfill (BARC 27). P-03-0137*

BMT Entech Inc. (2009) Proposed plan for the Beaver Dam Road landfill (BARC 27). D-19-0010

BMT (2014) Beaverdam road landfill: Biowall performance monitoring plan. Updated December 2014

BMT Designers & Planners Inc. (2016) Three-year performance review report: October 2013 – September 2016 for the permeable reactive barrier remedy at the Beaverdam Road landfill (BARC 27)

Bradley PM, Chapelle FH. (1998) Microbial mineralization of VC and DCE under different electron accepting conditions. Anaerobe 4:81-87

Bertolino SM, Melgaço LA, Sá RG, Leão VA (2014) Comparing lactate and glycerol as a single-electron donor for sulfate reduction in fluidized bed reactors. Biodegradation 25:719-733

Burris DR, Campbell TJ, Manoranjan VS (1995) Sorption of trichloroethylene and tetrachloroethylene in a batch reactive metallic iron-water system. Environ Sci Technol 29:2850-2855

Chiao FF, Currie RC, McKone TE (1994) Intermedia transfer factors for contaminants found at hazardous waste sites: Trichloroethylene (TCE). A Research Report of the Department of Environmental Toxicology, University of California, Davis, USA. Prepared for the Office of Scientific Affairs, the Department of Toxic Substances Control (DTSC) and the California Environmental Protection Agency in Support of the CalTOX Model. http://www.dtsc.ca.gov/AssessingRisk/Upload/pce.pdf

Chiu WA, Jinot J, Siegel Scott C, Makris SL, Cooper GS, Dzubow RC, Bale AS, Evans MV, Guyton KZ, Keshava N, Lipscomb JC, Barone Jr. S, Fox JF, Gwinn MR, Schaum J, Caldwell JC (2013) Human health effects of trichloroethylene: Key findings and scientific issues. Environ Health Persp 121(3):303-311

da Silva GP, Mack M, Contiero J (2009) Glycerol: A promising and abundant carbon source for industrial microbiology. Biotechnol Adv 27(1):30-39

Davis JW, Odom JM, DeWeerd KA, Stahl DA, Fishbain SS, West RJ, Klecka GM, DeCarolis JG (2002) Natural attenuation of chlorinated solvents at Area 6, Dover Air Force Base: Characterization of microbial community structure. J Contam Hydrol 57:41-59

Emde R, Swain A, Schink B (1989) Anaerobic oxidation of glycerol by *Escherichia coli* in an amperometric poised-potential culture system. Appl Microbiol Biot 32(2):170-175

Erto A, Lancia A, Bortone I, Di Nardo A, Di Natale M, Musmarra D (2011) A procedure to design a permeable adsorptive barrier (PAB) for contaminated groundwater remediation. J Environ Manage 92:23-30

Farrell, J (1996) *Proceedings*, Fifth World Congress of Chemical Engineering, San Diego, CA, July 14-18, 1996; Paper 63a

Farrell J, Kason M, Melitas N, Li T (2000) Investigation of the long-term performance of zero-valent iron for reductive dechlorination of trichloroethylene. Environ. Sci. Technol 34:514-521

Federal Remediation Technologies Roundtable (FRTR) (2003) Abstracts of Remediation Case Studies Volume 7. Cincinnati, OH: U.S. EPA/National Service Center for Environmental Publications

FRTR (2010) Federal Remediation Technologies Roundtable meeting, Arlington, VA, May 13, 2010. https://frtr.gov/pdf/meetings/may10/SP_2010_FRTR_Mtg_Summary.pdf

Freeborn RA, West KA, Bhupathiraju VK, Chauhan S, Rahm BG, Richardson RE, Alvarez-Cohen L (2005) Phylogenetic analysis of TCE-dechlorinating consortia enriched on a variety of electron donors. Environ Sci Technol 39:8358-8368

Freedman DL, Gossett JM (1989) Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl Environ Microb 55(9):2144-2151

Gilbert O, Cortina JL, de Pablo J, Ayora C (2013) Performance of a field-scale permeable reactive barrier based on organic substrate and zero-valent iron for in situ remediation of acid mine drainage. Environ Sci Pollut R 20:7854-7862

Groundwater Services, Inc. (GSI) (2001) Final report: Mulch biowall and surface amendment pilot test, Air Force Center for Environmental Excellence, Contract number: F41624-97-C-8020, CDRL Number A006

GSI (2004) Report for full-scale mulch wall treatment of chlorinated hydrocarbon-impacted groundwater. Houston, TX: Groundwater Services, Inc. Contract Number: F41624-97-C-8020

He YT, Wilson JT, Wilkin RT (2008) Transformation of reactive iron minerals ina permeable reactive barrier (biowall) used to treat TCE in groundwater. Environ Sci Technol 42:6690-6696

Hendrickson ER, Payne JA, Young RM, Starr MG, Perry MP, Fahenstock S, Ellis DE, Ebersole RC (2002) Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. Appl Environ Microb 68(2):485-495

Henry BM, Hartfelder T, Goodspeed M, Gonzales JR, Haas PE, Oakley D (2003) Permeable mulch biowall for enhanced bioremediation of chlorinated ethenes. Seventh International Symposium of In Situ and On-Site Bioremediation, Orlando, Florida

Heron G, Christensen TH, Enfield CG (1998) Henry's law constant for trichloroethylene between 10 and 95 °C. Environ Sci Technol 32:1433-1437

Hughes L, Robertson S (2002) Glycerol: SIDS initial assesment report for SIAM 14. United Nations Environmental Programme Publications

Ingram DT, Callahan MT, Ferguson S, Hoover DG, Chiu PC, Shelton DR, Millner PD, Camp MJ, Patel JR, Kniel KE, Sharma M (2012) Use of zero-valent iron biosand filters to reduce Escherichia coli O157:H12 in irrigation water applied to spinach plants in a field setting. J Appl Microbiol 112(3):551-560

Jacoby WA, Blake DM, Watt AS. 1998. Remediation of trichloroethylene or perchloroethylene contamination. In: Meyers RA (ed) Encyclopedia Series in Environmental Sciences: Environmental Analysis and Remediation. Wiley Blackwell, USA, pp 4847-4873

Kassenga G, Pardue JH, Moe WM, Bowman KS (2004) Hydrogen thresholds as indicators of dehalorespiration in constructed treatment wetalnds. Environ Sci Technol 38(4):1024-1030

Kouznetsova I, Bayer P, Ebert M, Finkel M (2007) Modelling the long-term performance of zero-valent iron using a spatio-temporal approach for iron aging. J Contam Hydrol 90: 58-80

Lee CY, Cheng SZ, Chang CK, Lee FY, Liao CJ (2007) Simplified approach for rapid estimation of trichloroethylene adsorption onto soils. Pract Period Hazard Toxic Radioact Waste Manage 11(3):172-176

Lee C, Kim JY, Lee WI, Nelson KL, Yoon J, Sedlak DL (2008) Bactericidal effect of zero-valent iron nanoparticles on *Escherichia coli*. Environ Sci Technol 42(13):4927–4933

Liu Y, Majetich SA, Tilton RD, Sholl DS, Lowry GV (2005) TCE dechlorination rates, pathways, and efficiency of nanoscale iron particles with different properties. Environ Sci Technol 39:1338-1345

Maymó-Gatell X, Anguish T, Zinder SH. 1999. Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "*Dehalococcoides ethenogenes*" 195. Appl Environ Microb 65(7):3108-3113

National Institute for Occupational Safety and Health (NIOSH) (2015) Methanol: Systemic agent. http://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750029.html. Accessed 12 April 2017

Niño de Guzmán GT, Hapeman CJ, Millner PD, McConnell LL, Michaelson DE, Jackson D, Kindig D, Kjellerup B, Torrents A (2017) Using adaptive management to guide a trilateral cooperation between federal agencies, academia, and industry in remediating TCE with a permeable reactive barrier. Manuscript submitted for publication

Orth WS, Gillham RW (1996) Dechlorination of trichloroethene in aqueous solution using Fe^0. Environ Sci Technol 30:66-71

Ozturk Z, Tansel B, Katsenovich Y, Sukop M, Laha S (2012) Highly organic natural media as permeable reactive barriers: TCE partitioning and anaerobic degradation profile in eucalyptus mulch and compost. Chemosphere 89:665-671

Pant P, Pant S (2010) A review: Advances in microbial remediation of trichloroethylene (TCE). J Environ Sci 22(1):116-126

Pavolostathis SG, Jaglal K (1991) Desorption behavior of trichloroethylene in contaminated soil. Environ Sci Technol 25(2):274-279

Phillips DH, Van Nooten T, Bastiaens L, Russell MI, Dickson K, Plant S, Ahad JME, Newton T, Elliot T, Kalin RM (2010) Ten year performance evaluation of a field-scale zero-valent iron permeable reactive barrier installed to remediate trichloroethene contaminated groundwater. Environ Sci Technol 44:3861-3869

Poulsen TG, Yamaguchi T, Moldrup P, de Jong LW, Rolston DE (2000) Predicting volatile organic vapor sorption from soil specific surface area and texture. J Environ Qual 29:1642-1649

Qatibi AI, Bennisse R, Jana M, Garcia JL (1998) Anaerobic degradation of glycerol by *Desulfovibrio fructosovorans* and *D. carbinolicus* and evidence for glycerol-dependent utilization of 1,2-propanediol. Curr Microbiol 36(5):283-290

Schaerlaekens J, Mallants D, Šimûnek J, van Genuchten MTh, Feyen J (1999) Numerical simulation of transport and sequential biodegradation of chlorinated aliphatic hydrocarbons using CHAIN_2D. Hydrol Process 13:2847-2859

Tandoi V, DiStefano TD, Bowser PA, Gossett JM, Zinder SH (1994) Reductive dehalogenation of chlorinated ethenes and halogenated ethanes by a high-rate anaerobic enrichment culture. Environ Sci Technol 28:973-979

Tratnyek PG, Johnson TL, Scherer MM, Eykholt GR (1997) Remediating ground water with zero-valent metals: Chemical considerations in barrier design. Ground Water Monit R 17(4):108-114

USDA-ARS (2009) CERCLA proposed plan for the Beaver Dam Road landfill site. United States Department of Agriculture, Agricultural Research Service. June 2009

USDA-ARS (2012) *Beltsville Agricultural Research Center Screening Investigations of Potential Areas of Concern.* https://www.ars.usda.gov/ARSUserFiles/80000000/CERCLA/CERCLA-2012/AreasofConcern.pdf. *Accessed 12 May 2017*

US EPA (2001) Sources, emission and exposure to trichloroethylene (TCE) and related chemicals. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington Office, Washington, DC, EPA/600/R-00/099. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=21006. Accessed 12 May 2017

US EPA (2009) National primary drinking water regulations. U.S. Environmental Protection Agency, EPA/816/F/09/004. https://www.epa.gov/sites/production/files/2016-06/documents/npwdr_complete_table.pdf. Accessed 12 May 2017

US EPA (2016a) Trichloroethylene (tce): Chemistry and behavior. https://clu-in.org/contaminantfocus/default.focus/sec/trichloroethylene_(tce)/cat/Chemistry_and_Behavior/. Accessed 13 April 2017.

US EPA (2016b) Regional screening levels (RSLs)- Generic tables (May 2016). https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables-may-2016. Accessed 13 April 2017

US EPA (2016c) Regional screening levels (RSLs)- User's guide (May 2016). https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide-may-2016. Accessed 23 May 2017

Viana MB, Freitas AV, Leitão RC, Pinto GAS, Santaella ST (2012) Anaerobic digestion of crude glycerol: A review. Environ Technol Rev 1(1):81-92

Vogan JL, Focht RM, Clark DK, Graham SL (1999) Performance evaluation of a permeable reactive barrier for remediation of dissolved chlorinated solvents in groundwater. J Hazard Mater 68:97-108

Vogel TM, McCarty PL (1987) Rate of abiotic formation of 1,1-dichloroethylene from 1,1,1-trichloroethane in groundwater. J Contam Hydrol 1(3):299-308

Weber EJ (1996) Iron-mediated reductive transformations: Investigation of reductive mechanism. Environ Sci Technol 30(2):716-719

Weidemeier TH, Newell CJ, Rifai HS, Wilson JT (1999) Natural attenuation of fuels and chlorinated solvents in the subsurface. John Wiley & Sons, New York, pp 617

Wilkin RT, Puls RW, Sewell GW (2003) Long-term performance of permeable reactive barriers using zero-valent iron: geochemical and microbiological effects. Ground Water 41(4):493-503

Wu W, Kitagawa M, Taniguchi S, Jain MK (1998) Anaerobic dechlorination of perchloroethylene (PCE) in soil by a dechlorinating microbial consortium. J Ferment Bioeng 86(6):588-594

Yang X, Fan LT, Erickson LE (1995) Bio-wall technology: Conceptual design and analysis. Proceedings of the 10th Annual Conference on Hazardous Waste Research May 23-24, 1995. Kansas State University

Yang Y, Guo J, Hu Z (2013) Impact of nano zero valent iron (ZVI) on methanogenic activity and population dynamics in anaerobic digestion. Water Research 47(17):6790-6800

Yu S, Dolan ME, Semprini L (2005) Kinetics and inhibition of reductive dechlorination of chlorinated ethylenes by two different mixed cultures. Environ Sci Technol 39:195-205

Zabetakis KM, Niño de Guzmán GT, Torrents A, Yarwood S (2015) Toxicity of zero-valent iron nanoparticles to a trichloroethylene-degrading groundwater microbial community. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering 50(8):794-805

Zhang Y, Hu M, Li P, Wang X, Meng Q (2015) Analysis of trichloroethylene removal and bacterial community function based on pH-adjusted in an upflow anaerobic sludge blanket reactor. Appl Microbiol Biotechnol 99:9289-9297

Chapter 3: Presence of organohalide-respiring bacteria in and around a permeable reactive barrier at a trichloroethylene-contaminated Superfund site

Remarks on the biodegradation of TCE

TCE was previously thought to be resistant to biodegradation until a discovery by Maymó-Gatell, et al. (1997) described an organism capable of fully and completely dechlorinating tetrachloroethylene (PCE) (Maymó-Gatell et al. 1997; Hendrickson et al. 2002). *Dehalococcoides ethenogenes* strain 195 (now *D. mccartyi* strain 195), was genetically determined to be a Gram-positive eubacteria and a loose relation of *Clostridium butyricum* (Maymó-Gatell et al. 1997; Hendrickson et al. 2002; Seshadri et al. 2005; Löffler et al. 2013). *D. mccartyi* strain 195 only uses H_2 as its electron donor and chlorinated compounds as electron acceptors (Seshadri et al. 2005). Other strains have since been identified along with the functional genes necessary for dechlorination (Maymó-Gatell et al. 1997; He et al. 2005; Cupples et al. 2004; Lee et al. 2006; Sung et al. 2006). Lee, et al. (2006) determined that while expression of these genes could be used as a biomarker to determine bacterial activity they could not be used to correlate contaminant concentration and dechlorination rate.

Other studies investigating the microbial populations of contaminated areas have shown that while there may be a particular species or genus specially suited for the degradation of the contaminant of concern, often they rely on other organisms to provide resources they themselves cannot make or find in their current surroundings; this can also include relying on biostimulation efforts in order to encourage proliferation (Paliy 2013; Kang and Doty 2014; Zhang et al. 2015; Davis et al. 2002;

Wiedemeier et al. 1996; Atashgahi et al. 2017; Yan et al. 2016; Yi et al. 2012; Zhou et al. 2002). Bioaugmentation of contaminated areas with commercially available consortia such as KB-1 (SiREM, Guelph, Ontario, Canada) and SDC-9 (RNAS Remediation Products, Brooklyn Center, MN) have utilized the methanogens, acetogens, and sulfate reducers to support the other workhorses, *Geobacter* and *Dehalococcoides* for remediation efforts (Haest et al. 2010; Vainberg et al. 2009). Methanogens, acetogens, and sulfate-reducing bacteria themselves have demonstrated an ability to partially degrade TCE (Maymó-Gatell et al. 1997; Rahm et al. 2006; Dugat-Bony et al. 2012).

It is important to remember that these populations are complex communities that interact with complex environments and that while the interaction between these two systems can be predicted to a certain extent, the final outcome may be different than anticipated due to changes in external factors (i.e. weather, hydrology). Bioaugmentation, even with the re-introduction of the native microbial community, may take more than one event to be successful. In that regard, running small bioreactors under the same environmental conditions as the site in question with different bioaugmentation communities and biostimulation circumstances can provide an understanding of the probable pitfalls to be faced in the field.

The findings from this work have been submitted to the *Journal of Applied Microbiology and Biotechnology*.

Abstract

Trichloroethylene (TCE) is one of the most common groundwater contaminants in the United States and continues to be difficult to clean up due to its physical and chemical properties. TCE and several of its degradation products were detected in the groundwater of the Beaver Dam Road Landfill site (Beltsville, MD) at concentrations above accepted maximum contaminant levels. A permeable reactive barrier (i.e. biowall) was installed to remediate the groundwater. Microbial infiltration and colonization of the biowall with native bacteria was expected to take place subsequently. An array of molecular biological tools was applied to survey the native microbial community for presence of dechlorinating microorganisms at the site. Microorganisms belonging to methanogens, acetogens, sulfate-reducing bacteria, and chlorinated aliphatic hydrocarbon-metabolizing bacteria were identified thus making way for the application of the native microbial populations in the biowall bioaugmentation efforts. Additionally, molecular approaches were used to monitor continuously-fed column reactors containing saturated biowall material spiked with a commercially-available *Dehalococcoides*-containing culture, SDC-9 with or without zero-valent iron (ZVI) shavings. The column without ZVI had the highest abundance of *Dehalococcoides* spp. (2.7 x 10^6 cells/g material, S.D. = 3.8 x 10^5 cells/g material), while the addition of ZVI did not impact the overall population. Therefore, if ZVI would be applied as a biowall material amendment, biostimulation would not be required to maintain a *Dehalococcoides* population. However, the addition of ZVI and biostimulation did change the *Dehalococcoides* strain ratios. These experimental

results will be utilized in future remediation and/or biowall expansion plans to fully employ the natural resources at the study site.

Introduction

Trichloroethylene (TCE) is one of the most common groundwater contaminants in the United States and was detected at 57% of the National Priorities List (NPL) sites in 2015 (ATSDR 2015; ATSDR 2017). TCE is of particular concern because of its classification as a human carcinogen and its detrimental effects to the nervous system (ATSDR 2011). Moreover, vinyl chloride (VC), a degradation product of TCE, is 2.5 times more toxic than TCE and is also classified as a human carcinogen (ATSDR 2006; US EPA 2009). TCE remediation is challenging due to the high volatility of TCE and propensity to form a dense non-aqueous phase liquid in an aquifer (Chiao et al. 1994; US EPA 2016; Jacoby et al. 1998). TCE was detected in the groundwater of the Beaver Dam Road Landfill site (Beltsville, MD) at a concentration two levels of magnitude or more above the maximum contaminant level (MCL) of 5 ppb (USDA-ARS 2012; US EPA 2009). With the site's inclusion in the CERCLA (Superfund) Program, lengthy site investigations and a feasibility study were carried out to determine the extent of the contamination. Based on the findings from the feasibility study, a biowall was selected as the remedial action (BMT Entech Inc., 2009; Niño de Guzmán et al. 2018a, b; USDA-ARS, 2012; US EPA, 2009). The composition of the biowall was determined based on laboratory experiments resulting in a mixture of 30% sand and 70% organic material mixture (Nino de Guzman et al. 2018b). The organic component was made up of mulch and compost (ratio 4;3) (Nino de Guzman et al. 2018b). Zero-valent iron (ZVI) shavings and glycerol were considered as potential amendments but were not added at the time of installation in 2013 (Nino de Guzman et al. 2018b).

Biowalls are a type of green technology installed to remove or reduce groundwater contamination, usually to resolve issues where the source cannot be directly managed and the source lifespan is unknown (Powell et al. 1998). Biowalls are active barriers and filters, where fill-materials comprised of organic matter and other materials trap and subsequently aid in degrading sorbed contaminants. ZVI is a strong reducing agent that has been used in a number of studies for the treatment of chlorinated organic compounds without the help of additional materials (Chen et al. 2011; Farrell et al. 2000; Orth and Gillham 1996). Though ZVI was not installed with the biowall at this time, it is still under consideration for use in the future. As an open system, microbial infiltration and colonization into this porous structure was expected. While ZVI is a powerful tool for remediation, studies have shown that ZVI also has the potential to inactivate or kill bacteria (Gu et al. 1999; Wilkin et al. 2003; Ingram et al. 2012; Lee et al. 2008; Diao and Yao 2009; Chen et al. 2011). To avoid this, particle size, dose, and some form of biostimulation should be considered when employing ZVI.

Microbial reductive dechlorination is an important conduit for TCE dechlorination. Activity measurements from on-site experiments have shown that the rates of dechlorination are dependent on the abundance of dechlorinating bacteria, soil properties, and the mass loading of reactive minerals (Dong et al. 2009). Therefore, it is advantageous to utilize this naturally occurring process and employ the native microbial population to improve the biowall activity by promoting simultaneous biotic and abiotic degradation inside the structure (Dong et al. 2009).

Previous studies have utilized culture-dependent and culture-independent methods based on the 16S rRNA gene such as sequencing and quantitative PCR (qPCR) to characterize native microbial populations in groundwater and other sediment systems (Davis et al. 2002; Semprini et al. 1997; Holliger et al. 1993; Da Silva et al. 2008; Fung et al. 2007). The discovery of *Dehalococcoides mccartyi* strain 195 (formerly *D. ethenogenes* strain 195) and its ability to reductively dechlorinate TCE to vinyl chloride (VC) and ethene was a benefit since TCE was previously considered as recalcitrant to biodegradation (Hendrickson et al. 2002; Maymó-Gatell, et al. 1997; Seshadri et al. 2005; Löffler et al. 2013). Other dechlorinating bacteria and functional genes targeting chlorinated ethenes have since been identified (He et al. 2005; Cupples et al. 2004; Lee et al. 2006; Sung et al. 2006; Krajmalnik-Brown et al. 2004). *D. mccartyi* strains 195 and FL2 contain the functional gene *tceA*, which encodes for a TCE reductive dehalogenase that transforms TCE to VC, while strains VS and GT utilize the gene *vcrA* for degradation of TCE to ethene; D. mccartyi strain BAV1 employs the gene *bvcA* to reduce VC to ethene (Johnson et al. 2005; He et al. 2003; Lee et al. 2006; Krajmalnik-Brown et al. 2004; Ritalahti et al. 2006). These genes can be used as biomarkers to determine the potential for microbial reductive dechlorination activity in a site (Rowe et al. 2012; Heavner et al. 2018; Dugat-Bony et al. 2012; Ritalahti et al 2006). Bioaugmentation of TCE-contaminated sites with *Dehalococcoides* spp. and other microbial consortia containing organohalide-respiring organisms has shown great success especially in conjunction with biostimulation efforts (McDonald et al. 2012; Gilmore et al. 2012). In some bioaugmentation strategies, native microorganisms were isolated and reintroduced into the contaminated area to enhance the degradation process (Hood et al. 2008; Lendvay et al. 2003; Lee et al. 2012). The success of these efforts depended on the makeup of the targeted population, the inherent degradation capability of the population, and the cultivation or biostimulation efforts accompanying the bioaugmentation (Hood et al. 2008). Other studies successfully used a commercially available culture for degradation with or without biostimulation (Harkness et al. 1999; Ellis et al. 2000; Lee et al. 2010; Major et al. 2002)

The objectives of this study were to (1) conduct a survey of the native soil microbial community to identify microbial TCE degradation clusters and the potential presence of *Dehalococcoides* species to support bioremediation efforts using native species and (2) to determine if *Dehalococcoides* spp. could survive ZVI amendment in a mock-up of the biowall.

In this study, environmental samples were collected from areas showing known presence or absence of dechlorination activity for chlorinated solvents. Soil cores were collected from an uncontaminated control location, and two areas upstream of the biowall with a high or moderate concentration of TCE. Fill-material samples from the biowall were collected in 2015, two years after its installation. Also, the habitability of the biowall structure was investigated through a series of flow-through column experiments to determine if biostimulation was necessary to ensure the survival of the culture inside the biowall.

Materials and Methods

Samples Collection

Soil samples were collected in September 2013 from three locations upstream of the biowall that were undisturbed by the installation process (Figure 2-1). The characteristics of these sites were: 1) Remedial Investigation well 4 (MW4): no detected TCE contamination (method detection limit = 0.3 μg/L; BMT Designers and Planners 2015); 2) approximately 30 m north of Biowall Well 4 (nBW4): low/moderate TCE contamination (260 ng/L); 3) Remedial Investigation well 6 (MW6): high TCE contamination (260-512 ng/L). Saturated soil cores (0.9 m long, approximately 5 cm diameter) were collected using a Geoprobe soil borer (Geoprobe Systems, Salina, KS). The cores were encased in plastic sheaths, which were tightly wrapped with pallet plastic wrap and kept on ice until transport to the laboratory, where they were frozen until analysis. DNA was extracted from 5 cm sections from the middle of the saturated zone: 3.7 to 4 m below ground surface (bgs) at MW4, 3.0 to 3.4 m bgs at nBW4, and 4.0 to 4.3 m bgs at MW6.

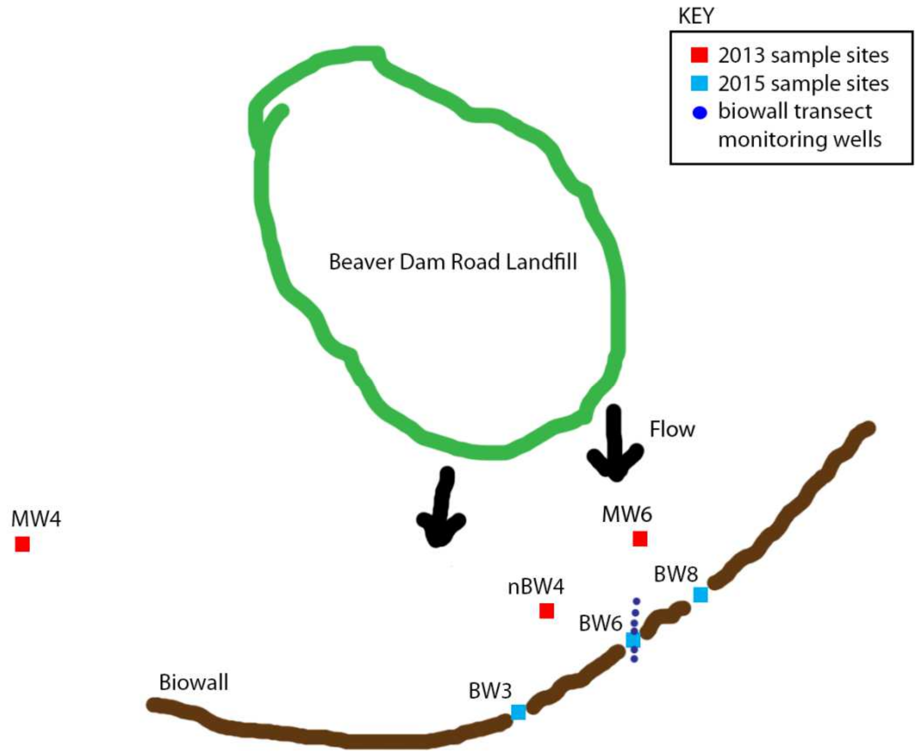


Figure 2-1: Map of the study site.

Biowall samples were collected in May 2015 from three locations within the biowall: A) BW3 with low/moderate TCE contamination (260 ng/L); B) BW6 with high TCE contamination (260–512 ng/L); C) BW8 with moderate contamination (130–260 ng/L) (Figure 2-1). The samples were collected using a 15.2 cm hand auger to penetrate the fill-material and collect the saturated samples. The cores were deposited on clean garbage bags, then wrapped up and secured with duct tape. At BW3, the saturated zone extended from 0.91 to 1.83 m bgs (due to the consistency, only the top and bottom 0.3 m were separated and saved). At BW6, the saturated zone extended from 1.22-1.83 m bgs (wrapped as two 0.3 m lengths). Lastly, at BW8 the saturated zone was encountered from 1.07 to 1.52 m bgs (wrapped as a single sample). These samples were kept on ice until transport to the laboratory, where they were frozen until analysis.

Flow-through column construction and operation

To determine if the biowall material had an environment that could support a microbial consortium containing *Dehalococcoides* spp., four flow–through columns were assembled using 2.5 by 20 cm flex-columns (Kimble Chase, Rockwood, TN) using material previously exposed to a 700 µg TCE bolus for approximately 160 days in batch reactors kept at 12°C (±2°C) (Nino de Guzman et al. 2018b). Each column was operated inside an anaerobic glove box to prevent oxygen intrusion (5% hydrogen, 5% carbon dioxide, 90% nitrogen) (Airgas, Allentown, PA) (Coy Laboratory Products, Grass Lake, MI). The columns were kept at room temperature (27°C) to enhance the rate of the processes compared to groundwater conditions (12-15°C). Reactors 1 and 2 were packed with a 4:3 ratio mulch/compost (M/C) mixture (average saturated material weight = 170 g with an average porosity of 40%), while Reactors 3 and 4 were packed with the M/C mixture containing 5 mL/L ZVI shavings (M/C+Fe) (aggregate size 8/50, density 6.62 g/mL) (Peerless Metal Powders and Abrasive, Detroit, MI). This ZVI dose was based on previous work described in Niño de Guzmán et al. (2018b). All reactors were spiked with 30 mL of the commercially available SDC-9 anaerobic consortium culture (*Dehalococcoides* ≥ 1×10^{11} cells/L, OD_{550} = 1.5; RNAS Remediation Products, Brooklyn Center, MN; Vainberg et al. 2009), equivalent to the approximate pore volume of the reactors. SDC-9 (here used as a surrogate for the native microbial community) contained at least one type of *Dehalococcoides mccartyi*, along with *Dehalogenimonas* spp., *Desulfovibrio* spp., *Desulfitobacterium* spp., sulfate-reducing bacteria, and methanogens (RNAS Remediation Products, Brooklyn Center, MN; Popat et al. 2010).

All reactors were continuously fed top-down at an average rate of 40 rpm for 107 days to mimic the groundwater flow rate at the site. Reactors 1 and 3 were fed with nitrogen-sparged sand-filtered groundwater (gw) collected from MW4 spiked with 600 ppm TCE. Reactors 2 and 4 were fed with TCE-spiked, nitrogen-sparged, modified RAMM media made with sand-filtered groundwater collected from MW4 (personal communication, CB&I; Shelton and Tiedje, 1984). The RAMM media consisted of three solutions, prepared separately: a 2 M (or 100X) phosphate buffer, a 100X mineral salts solution (53.0 g NH_4Cl, 7.5 g $CaCl_2 \bullet 2H_2O$, 10.0 g $MgCl_2 \bullet 6H_2O$, 2.0 g $FeCl_2 \bullet 4H_2O$ per liter), and a 1000X trace metal solution (5.0 g $MnCl_2 \bullet 4H_2O$, 0.05 g H_3BO_3, 0.05 g $ZnCl_2$, 0.03 g $CuCl_2$, 0.01 g $Na_2MoO_4 \bullet 2H_2O$, 0.5 g $CoCl_2 \bullet 6H_2O$, 0.05 g $NiCl_2 \bullet 6H_2O$, 0.05 g Na_2SeO_3 per liter plus approximately 1 mL of 38% HCl to keep metals suspended). These three solutions were mixed with the nitrogen-sparged sand-filtered groundwater collected from MW4 to a final concentration of 1X plus 1.2 g/L of $NaHCO_3$ and 1.0 g/L of yeast extract. At the conclusion of the experiment, each column was divided into three sections (6.7 cm long) and each section was separately transferred to sterile centrifuge tubes. The material of each section was homogenized before further analysis. The abundance of *Dehalococcoides* spp., *tceA*, *vcrA*, and *bvcA* was quantified in different sections of the columns.

DNA extraction

DNA was extracted from all *in-situ* samples (soil and biowall) using MoBio PowerMax Soil DNA isolation kit (QIAGEN, Inc., Germantown, MD) following the manufacturer's instructions using an average of 9.9 g (wet weight) of sample. This kit was also used to extract DNA from a 40 mL aliquot of the SDC-9 culture following the manufacturer's instructions. DNA was extracted from column reactors using MoBio PowerSoil DNA isolation kit (QIAGEN, Inc., Germantown, MD) using 0.25 g (wet weight) of sample following the manufacturer's instructions.

PCR and qPCR

A nested PCR reaction was used to target Bacteria and Archaea with the universal 16S rRNA primer set from the IDT ReadyMade primer series, 16S rRNA Forward and 16S rRNA Reverse (ReadyMade™ Primers, Integrated DNA Technologies, Inc., Coralville, IA) (Table 2-1). This PCR product was subsequently used as template (nested PCR) for evaluation of *Dehalococcoides* species using specific primers targeting three reductive dehalogenase enzymes (RDases) via their corresponding functional genes designated *tceA* (TCE RDase), *vcrA* (VC RDase), and *bvcA* (VC RDase) (Table 2-1). The reaction mix for all PCR reactions consisted of: 9.5 µL PCR-grade water (Integrated DNA Technologies, Inc., Coralville, IA), 12.5 µL DreamTaq Green PCR Master Mix (2X) (ThermoFisher Scientific, Waltham, MA), 1.0 µL of each 10 µM F/R primer, and 1.0 µL template (total volume of 25 µL). The PCR conditions for the universal primer set and the individual targets can be found in

Table 2-1. A Bio-Rad T100 thermal cycler (Bio-Rad, Hercules, CA) was used for PCR.

Table 2-1: PCR primers and thermal cycler conditions.

Target	Primer Name	Sequence	Amplicon Length (bp)	Cycler Conditions	Source
Bacteria, Archaea	16S rRNA For	5'- AGA GTT TGA TCC TGG CTC AG	~1500	95°C (3 min), [95°C (45 sec), 50°C (1 min), 72°C (2 min)] x 35 cycles, 72°C (10 min)	Integrated DNA Technologies, Inc.; Weisburg et al. 1991
	16S rRNA Rev	5'- ACG GCT ACC TTG TTA CGA CTT			
Dehalococcoides spp.	Dhc728F	5'-AAG GCG GTT TTC TAG GTT GTC AC	444	95°C (3 min), [95°C (45 sec), 50°C (1 min), 72°C (2 min)] x 35 cycles, 72°C (10 min)	Yan et al. 2009; Loeffler et al. 2000
	Dhc1172R	5'-CGT TTC GCG GGG CAG TCT			
tceA	Deth_tceAD2F	5'-GTG GGA GGG TAC GCC TGA AG	586	95°C (5 min), [95°C (1 min), 50°C (1 min), 72°C 2 min)] x 30 cycles, 72°C (5 min)	Nishimura et al. 2008
	Deth_tceAD4R	5'-TAG GGA ACC CTT GGT GTT G			
vcrA	vcrAB_F	5'-CTA TGA AGG CCC TCC AGA TGC	1482	94°C (12 min), [94°C (1 min), 50°C (45 sec), 72°C (2 min)] x 30 cycles, 72°C (12 min)	Muller et al. 2004; Holmes et al. 2006
	vcrAB_R	5'-GTA ACA GCC CCA ATA TGC AAG T			
bvcA	bvcAF	5'-TGC CTC AAG TAC AGG TGG T	839	94°C (12 min), [94°C (1 min), 50°C (45 sec), 72°C (2 min)] x 30 cycles, 72°C (12 min)	Krajmalnik-Brown et al. 2004; Holmes et al. 2006
	bvcAR	5'-ATT GTG GAG GAC CTA CCT			

qPCR was applied to quantify the abundance of each target. This information was subsequently converted into the number of bacteria in each environmental sample (Figures 2-2 and 2-3). Previously published studies have identified a single 16S rRNA gene copy per cell in the *Dehalococcoides* genome, as well as single copies of the *tceA*, *vcrA*, and *bvcA* functional genes in each cell (He et al. 2003; Behrens et al. 2008). Therefore, the number of gene copies equal the number of bacterial cells detected in this study. *Dehalococcoides* species and the functional genes *tceA*, *vcrA*, and *bvcA* were targeted to determine the resident biological TCE degradation potential. The qPCR primers used to target *Dehalococcoides*, *tceA*, *vcrA*, and *bvcA* are listed in Table 2-2. These reactions were initially tested using a PCR thermal cycler to confirm the amplification conditions. Each reaction (triplicate) contained 8.0 µL PCR-grade water, 12.5 µL iTaq Universal SYBR Green Supermix (2X) (Bio-Rad, Hercules, CA), 1.25 µL of each 10 µM F/R primer, and 2.0 µL template (total volume of 25 µL). Using an Eppendorf realplex^2 thermal cycler (Eppendorf, Hauppauge, NY) the reaction conditions were as follows: 50°C for 2 min, 95°C for 10 min, [95°C for 15 sec, 60°C for 1 min] for 40 cycles (Da Silva et al. 2008). PCR was used in conjunction with the qPCR primers listed in Table 2 to isolate the qPCR amplicons for *Dehalococcoides* and the three functional genes. These PCR products were then run on a 1.5% low-melt agarose gel at 60V for 2 hours at 5°C. A Wizard SV Gel and PCR Clean-Up kit (Promega Corporation, Madison, WI) was used to process the gel products before each was quantified on a NanoDrop. Amplicon length was verified using PCR and gel electrophoresis. All of the qPCR standard curves were prepared using DNA from the culture SDC-9 as the starting material. The standard curve concentration ranges were as follows: *Dehalococcoides,* 4.7 x10^2 to 4.7 x10^9 gene copies/µL, *tceA,* 6 x10^2 to 6 x10^9 gene copies/µL, *vcrA,* 6.7 x10^2 to 6.7 x10^{10} gene copies/µL, and *bvcA,* 3.2 x10^0 to 3.2 x10^9 gene copies/µL. SDC-9 was used as a positive control and PCR-grade water was used as a negative control in all PCR and qPCR reactions.

Table 2-2: qPCR primers

Target	Primer Name	Sequence	Amplicon Length (bp)	Cycler Conditions	Source
Dehalococcoides spp.	Dhc1200F	5'-CTG GAG CTA ATC CCC AAA GCT	72	50°C (2 min), 95°C (10 min), [95°C (15 sec), 60°C (1 min)] x 40 cycles	Da Silva et al. 2008; He et al. 2003
	Dhc1271R	5'-CAA CTT CAT GCA GGC GGG			
tceA	tceA1270F	5'-ATC CAG ATT ATG ACC CTG GTG AA	67	50°C (2 min), 95°C (10 min), [95°C (15 sec), 60°C (1 min)] x 40 cycles	Da Silva et al. 2008; Johnson et al. 2005
	tceA1336R	5'-GCG GCA TAT ATT AGG GCA TCT T			
vcrA	vcrA_Fwd	5'-CTC GGC TAC CGA ACG GAT T	65	50°C (2 min), 95°C (10 min), [95°C (15 sec), 60°C (1 min)] x 40 cycles	Lee et al. 2006
	vcrA_Rev	5'-GGG CAG GAG GAT TGA CAC AT			
bvcA	bvcAF	5'-TGC CTC AAG TAC AGG TGG T	839	94°C (12 min), 30 cycles [94°C (60 s), 50°C (45 s), and 72°C (120 s)], 72°C (12 min)	Krajmalnik-Brown et al. 2004; Holmes et al. 2006
	bvcAR	5'-ATT GTG GAG GAC CTA CCT			

Population analysis

The universal and nested Dhc728F/1171R PCR products were sequenced by the Bioanalytical Services Laboratory at the Institute of Marine and Environmental

Technology (IMET) at the University of Maryland Baltimore County (Baltimore, MD) using an Illumina MiSeq platform (Illumina, Inc., San Diego, CA). Each run was spiked with 5% PhiX DNA (Illumina, Inc., San Diego, CA) control library to improve the run quality (personal communication, IMET). Initial data analysis was conducted using the MiSeq Reporter software (Illumina, Inc., San Diego, CA) in conjunction with a 16S rRNA gene database (https://greengenes.lbl.gov) to identify the reads (Table 2-3). Each read was run through the NCBI Taxonomic Database (https://www.ncbi.nlm.nih.gov/taxonomy) to verify taxonomic identification. The reads that were incorrectly classified and/or could not be defined within Family taxonomic level, were eliminated. The Family taxonomic level was the lowest common and reliable denominator used across all samples since not all samples were defined by Illumina beyond this point. While a 1.0% cutoff value has often been applied in studies using Illumina sequencing to exclude members with low representation (Zhang et al. 2012), this restriction eliminated approximately 30% of the population. Instead, a cutoff value of 0.1% was applied to each sample population. This resulted in exclusion of approximately 3% of the population in the biowall samples and approximately 1.5% of the population at MW6. A preliminary list of microorganisms was compiled from a literature search to screen each sample for the presence of dechlorinating bacteria or those affiliated with the dechlorination/dehalogenation process (Table 2-4/Supplementary Data, Table S1).

Table 2-3: Evaluation of bioinformatics raw data

Sample	Illumina Sample Information			Total Hits [(a)]	Total No. of Families Identified [(a)]	Total Hits >0.1% [(a)(b)]	Total No. of Families >0.1% [(a)(b)]
	Total Reads	Reads Passing QF	% Reads Passing QF				
MW6	390,378	311,159	79.7	45,146	181	44,450	101
BW6 shallow	62,410	52,657	84.4	30,586	195	29,700	106
BW6 deep	139,842	118,160	84.5	69,728	211	67,709	106
BW8	26,552	22,309	84.0	12,762	189	12,348	105
BW3 shallow	68,677	58,370	85.0	38,055	205	36,750	95
BW3 deep	42,591	35,820	84.1	20,996	198	20,292	104
SDC9	22,433	19,621	87.5	17,271	83	17,099	18

QF = Quality Filtering; a = Post-filtering for taxonomic accuracy using NCBI Taxonomy Database (**https://www.ncbi.nlm.nih.gov/taxonomy**), samples with incomplete taxonomy were eliminated; b = Only includes hits above 0.1% at the Family taxonomic classification level.

Statistical analysis

The qPCR results from the soil, biowall, and reactor samples were analyzed using a one-way ANOVA combined with the Kruskal-Wallis test and followed by a Dunn's post test, for multi-group comparison (GraphPad Prism software, Version 5.01, GraphPad Software, Inc., La Jolla, CA; GraphPad Prism 2017; McDonald 2014;). The Kruskal-Wallis test is a nonparametric test that determines if there is a statistically significant difference between three or more groups, while Dunn's post test determines which of the groups are significantly different from each other.

Results

Evaluation *Dehalococcoides, tceA, vcrA,* and *bvcA* presence in environmental samples

Dehalococcoides were present in all of the 2013 soil samples and in one of the 2015 biowall samples, BW6 (Figure 2-2a). The average number of *Dehalococcoides* present in the 2013 soil samples were 104.6 ± 0.6 cells/g soil whereas at BW6 this number was slightly higher, 182.9 ± 58.5 cells/g soil. An evaluation of 5 cm core segments did not reveal a significant difference in the *Dehalococcoides spp.* population as the depth increased within the 30 cm saturated-zone core section examined (data not shown).

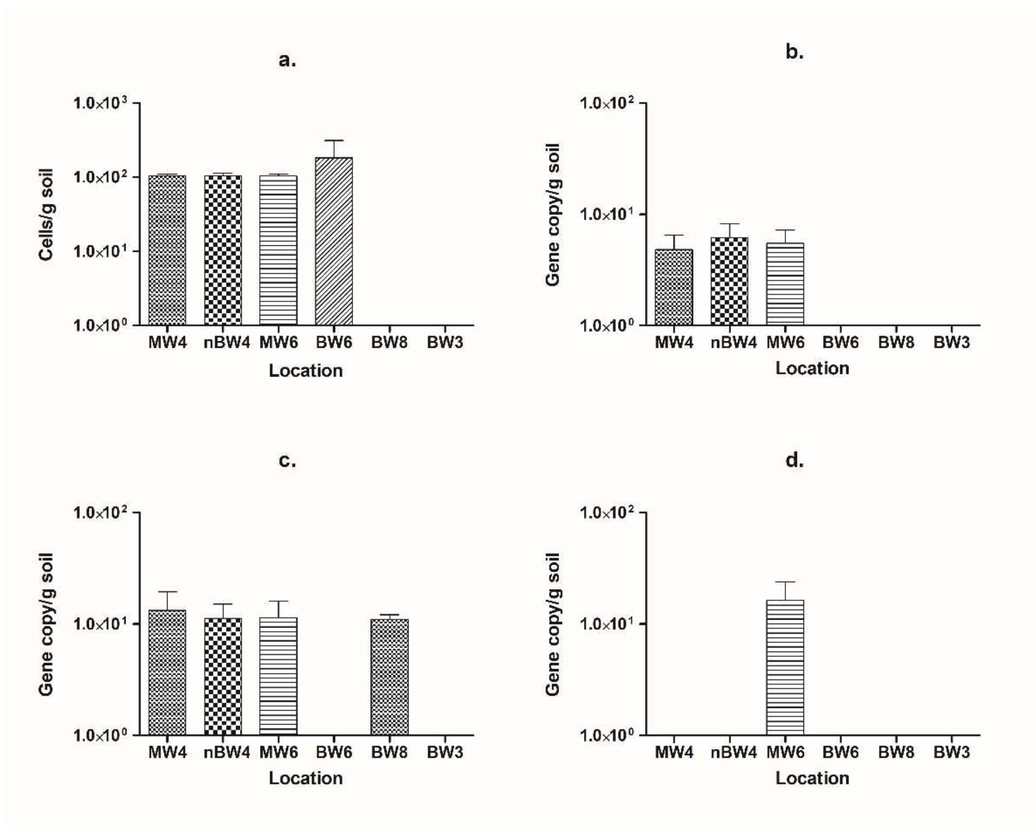


Figure 2-2: ***Dehalococcoides* spp. and RDase functional genes detected in environmental samples MW4, nBW4, MW6, and BW6. a) *Dehalococcoides* spp. (based on 16S rRNA gene), b) *tceA* gene (functional gene), c) *vcrA* gene (functional gene), and d) *bvcA* (functional gene).**

The RDase gene *tceA* was detected in all 2013 soil samples with approximately 5.5 ± 0.7 gene copies/g soil. This indicates that despite the differences in TCE concentration, *Dehalococcoides* strains (namely) 195 and FL2 were present in similar quantity at all of the sampling locations. *tceA* was found in approximately 4.5-6% of the population at nBW4, MW6, and MW4. Likewise, *vcrA* was detected in all soil samples (approximately 11.3 to 13.3 gene copies/g soil) with no significant difference in abundance; this gene outnumbered *tceA* by approximately 2 to 1 (nBW4, MW6) and 2.7 to 1 (MW4). Relative to the quantity of *Dehalococcoides*, *vcrA* was found in approximately 11.4% of the population (namely linked to *Dehalococcoides* strains VS and GT) (Figure 2-2c). *vcrA* was also detected in the BW8 sample (approximately 11.0 gene copies/g soil) though *Dehalococcoides* was not detected at this site using these methods. It is possible that the biowall samples themselves interfered with the DNA extraction protocols. The functional gene *bvcA* was only detected at MW6 and at a ratio of 1.4 to 1 versus *vcrA* and 2.95 to 1 versus *tceA*. The abundance of *bvcA* (16.3 gene copies/g soil) relative to the overall quantity of *Dehalococcoides* indicates that strain BAV1 comprised approximately 15.6% of the population.

Previous work has shown that the concentration of *Dehalococcoides* 16S rRNA and *vcrA* have a strong positive correlation with Fe^{2+}, pH, low oxidation/reduction (redox) potential, and VC and ethene concentrations, and a strong negative correlation with sulfate concentration (van der Zaan et al. 2010; Johnson et al. 2005; Lee et al. 2006). Likewise, *tceA* and *bvcA* genes were found to be positively correlated with a high redox potential, and Fe^{2+}, sulfate, and nitrate concentrations (van der Zaan et al. 2010; Johnson et al. 2005; Lee et al. 2006). Nitrate and nitrite were measured in the field for the first year after the biowall was installed before being discontinued as neither was detected in the groundwater samples (BMT Designers and Planners 2015). Ferrous iron (Fe^{2+}, filtered) continues to be measured at the biowall and transect wells, though not at MW4, nBW4, or MW6 as per the monitoring plan. At the time of collection in May 2015, the Fe^{2+} concentrations at BW3, BW6, and BW8 were 93 mg/L, 63 mg/L, and 50 mg/L (March 2015), respectively; since then, the concentrations have increased to 150 mg/L, 110 mg/L, and 160 mg/L, respectively (Niño de Guzmán et al. 2018b). In September 2016, Fe^{2+} was measured at MW6 at 19 mg/L (BMT Designers and Planners 2016). No significant differences were found between the Fe^{2+} concentrations present at each site; this concentration does not appear to have affected the quantity of any of the genetic targets. With respect to pH, there was no statistical difference between the biowall wells; the average pH of the three biowall sampling locations was 6.27 ± 0.05. In terms of the contaminants of interest, field measurements at MW6 showed a consistently high (>400 µg/L) TCE concentration and comparatively low concentrations of DCE and VC (<12 µg/L); no ethene was detected (Niño de Guzmán et al. 2018b). Downstream, at BW6, the TCE concentration was much lower but more variable [0.33-36 µg/L] while the DCE and VC concentrations were higher, 66-250 µg/L and 1-44 µg/L, respectively (Niño de Guzmán et al. 2018b). Though the ethene concentration at BW6 has increased since the installation of the biowall it has remained relatively low, approximately 5 µg/L (Niño de Guzmán et al. 2018b). At BW3, no TCE, DCE, or VC was detected; ethene was only detected once (September

2016) at a concentration of 1.1 µg/L. At BW8, the TCE concentration varied between 0.4 µg/L (September 2016) and 41 µg/L (January 2016) though at the time the sample was taken, it was approximately 7.3 µg/L (Niño de Guzmán et al. 2018b). The DCE and VC concentrations at this same time period were 12 µg/L [0.32-65 µg/L] and 4.2 µg/L [0.34-16 µg/L], respectively. The ethene concentration at BW8 has steadily increased from 1.3-7.2 µg/L, the concentration at the time of collection being 3.7 µg/L (Niño de Guzmán et al. 2018b). There were no significant differences between the sites with respect to VC or ethene concentrations; the gradual decrease of VC concentration and the gradual increase of ethene concentration do not appear to have affected the *Dehalococcoides* 16S rRNA or *vcrA* concentrations. Regardless, the degradation of VC is of vital importance as it is the most toxic of the TCE degradation products.

The redox conditions within the biowall were measured using a silver/silver-chloride electrode and ranged from an ORP of [-124 to +156 mV] at BW3, [-76 to +278 mV] at BW6, and [-115 to +221 mV] at BW8. Sulfate was detected at MW6 at a concentration of 250 mg/L (September 2016); this analyte is not regularly measured at this location but was a regular analyte of the biowall and transect wells (BMT Designers and Planners 2016). At BW6, sulfate ranged from 33-110 mg/L, while at BW8 this was more variable (<0.3-110 mg/L); BW3 had a lower concentration, ranging from <0.3-45 mg/L (BMT Designers and Planners 2016). A series of monitoring wells transecting the biowall (Figure 2-1) showed a steady increase in ethene production, though the concentration of DCE and VC downstream of the biowall remained at approximately 200 μg/L and 25 μg/L, respectively (Niño de Guzmán et al. 2018b).

Identification and quantification of the microbial community composition

A description of the taxonomical hierarchy and frequencies for bacterial phyla in the soil and biowall samples is provided in Table 2-5/Table S2.

The objective of the microbial community survey was to determine if *Dehalococcoides* (Family *Dehalococcoidaceae*) were present at the site. *Dehalococcoidaceae* were discovered in all of the biowall samples but at quantities below the cutoff level (0.02-0.05%). They were not detected in the MW6 soil samples. This finding is not in agreement with qPCR, where *Dehalococcoides* were detected.

Microbes from the dehalogenation reference list (Table 2-4/Table S1) were compared to each sample's population. It was found that there was a 20-25% (family) taxonomic similarity between these lists representing approximately 33-50% of the total identified population (>0.1%) at BW8 and BW3 shallow, respectively. At MW6, the reference list comprised approximately 21% of the total population (19.8% taxonomic similarity). The MW6 sample shared approximately 73% Family similarity with its corresponding downstream site, BW6, and approximately 70-71 % similarity with the other biowall samples, BW3 (low/moderate TCE concentration) and BW8 (moderate TCE concentration).

Although *Dehalococcoidaceae* was either not detected or detected in very low quantity (<0.1 % cutoff), other important primary or supporting community members were identified such as, *Desulfovibrionaceae* (includes *Desulfovibrio* spp.), which were found in all samples above the 0.1 % cutoff value. *Desulfovibrio* has been shown to be crucial for the proliferation of *Dehalococcoides* by providing a steady supply of acetate, hydrogen, and corrinoid cofactors. The latter are vital for enzymes that support reductive dehalogenation and are only produced by few bacterial species (Atashgahi et al. 2017; Yan et al. 2016; Yi et al. 2012). Broad classifications, such as acetogens (Class *Clostridia*) and sulfate-reducing bacteria (including families such as *Desulfobulbaceae*, *Desulfovibrionaceae*, *Desulfobacteraceae*, *Peptococcaceae*, *Syntrophaceae*) were present in all samples. Acetogens represented approximately 10- 19% of the population, with the exception of BW6 (shallow) where this group only represented 0.7% of the population. Sulfate reducers represented approximately 2.9- 4.0% of the populations. Other organisms that have been identified as able to directly metabolize or co-metabolize chlorinated aliphatic hydrocarbons (CAH's) were found in all of the samples albeit in different quantities. At BW3, this CAH-degrading group made up approximately 11- 18% of the population while at BW6 and BW8 this group was found in only 1.5- 2.6% and 3.2% of the population, respectively. At MW6, this group was only found in 7.4% of the total population (>0.1%). Methanogens (such as *Methanosarcinaceae*, *Methnococcaceae*, *Methanomicrobiaceae*, *Methanosaetaceae*) were not abundant in soil or biowall samples, 0.2- 0.3% of the populations (not present at BW3 deep or MW6). The low number of methanogens could be due to the heterogeneous nature of the samples themselves as well as the highly unfavorable oxidation/reduction potential within the biowall (>-200 mV) at this time for their widespread presence; under laboratory settings, an oxidation/reduction potential below -300 mV is favorable (Davis et. al 2002; Wolfe 2011).

At least a 20% taxonomic similarity was found between the biowall/soil samples and the Families found in the literature-generated reference list compiled to identify "favorable" TCE-dechlorinating individuals/groups. This accounted for 20-50% of the total population (>0.1%).

Evaluation of the *Dehalococcoides* population in flow-through biowall columns

A one-way ANOVA comparing the operation of the four reactors revealed that at the conclusion of the experiment they did not have significantly different *Dehalococcoides* cell concentrations. All of the column populations increased from the initial SDC-9 bolus of $3x10^9$ cells. Reactor 1 with no amendment or specialized feed solution concluded with a *Dehalococcoides* population approximately 2.73 x 10^6 cells/g material (Figure 2-3a). While it was expected that the reactor without ZVI would contain a higher number of *Dehalococcoides* due to decreased environmental stress under the same feed conditions, it was not expected that Reactor 1 would also outperform Reactor 2, fed with RAMM media.

Though there was no significant variation in the quantity of the functional genes with respect to treatment, subtle differences were noted. The abundance of *tceA* in the reactors fed with RAMM media was greater than those only fed with groundwater

(Figure 2-3b), while Reactor 3 (MC+Fe) fed with groundwater contained the highest *vcrA* concentration (responsible for transforming TCE to ethene) (1.25 x 10^5 gene copies/ g material) (Figure 2-3c). This was unexpected as the partially defined, yeast-containing, modified-RAMM media was thought to provide a more favorable micro-nutrient content. Finally, the quantity of *bvcA* did not vary greatly between treatments though the concentration in Reactor 2 was higher than all the other reactors (Figure 2-3d). The ratios RDase content (*tceA*:*vcrA*:*bvcA*), were unique to each treatment. Reactor 1 heavily favored *bvcA* over both *vcrA* and *tceA* (1:24:84) while in Reactor 3 *bvcA* and *vcrA* were more evenly represented (1:41:40). Reactor 2 was slightly more evenly distributed in representation (1:0.4:6) while in Reactor 4 *bvcA* dominated (1:1:15). The two columns fed with groundwater had the greatest vcrA and bvcA content regardless of ZVI presence, while the RAMM-fed reactors were more skewed towards the presence of *bvcA*.

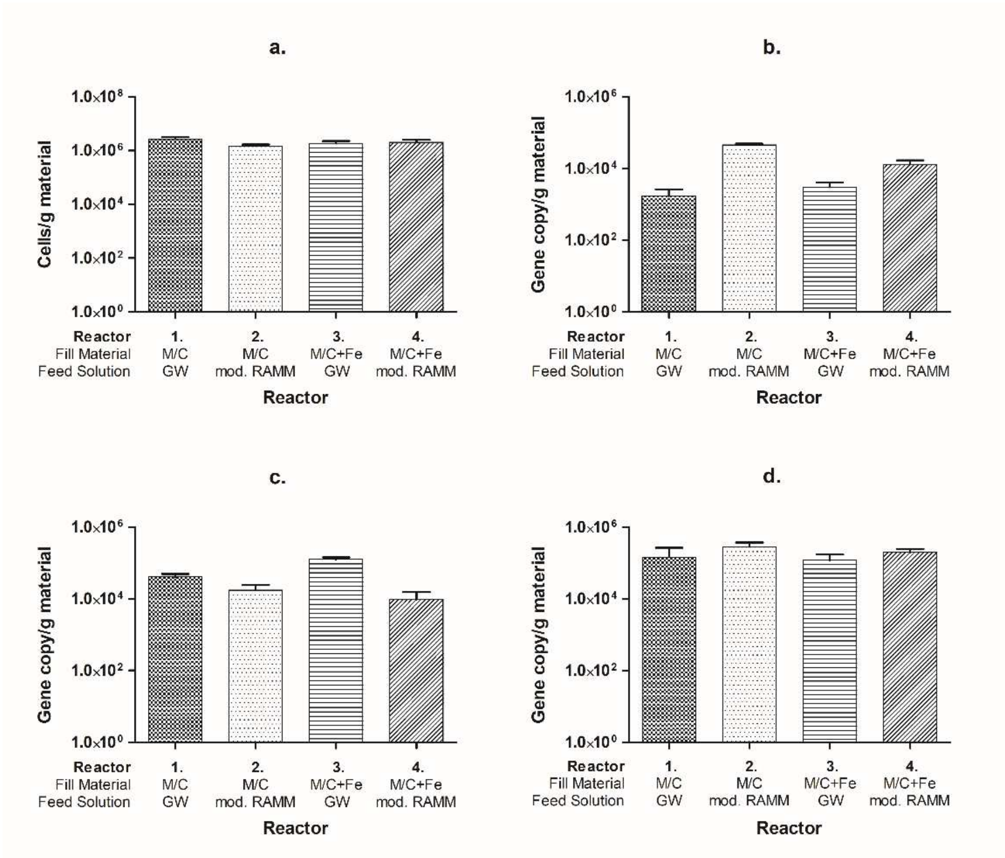


Figure 2-3: Functional genes *tceA*, *vcrA*, and *bvcA* quantified in samples from in column reactors. Reactor 1: M/C, groundwater fed; Reactor2: M/C, modified-RAMM media fed; Reactor 3: M/C+Fe, groundwater fed; Reactor 4: M/C+Fe, modified-RAMM media fed. a) *Dehalococcoides* spp.; b) *tceA* gene; c) *vcrA* gene; d) *bvcA* gene.

No significant differences were found with respect to the distribution of the *Dehalococcoides* population within the reactors, nor the tceA, vcrA, or bvcA functional genes. The functional genes were evenly distributed throughout the column reactors. Previous work with flow columns by Behren et al. (2008) showed that the abundance of *Dehalococcoides* and *tceA* decreased with increasing distance from the contaminant feed source, while the quantity of *vcrA* and *bvcA* were not affected by distance from the inflow of the column.

Discussion

While abiotic factors might have contributed to the degradation of TCE, the increasing ethene concentration suggests the involvement of the native microbial consortium containing *Dehalococcoides* in the biodegradation process (Haest et al. 2010; Rahm et al. 2006). *Dehalococcoides spp*. were found at the site, both in the soil and in the biowall. By also monitoring the functional genes responsible for the transformation of TCE to VC (*tceA*), TCE to ethene (*vcrA*), and VC to ethene (*bvcA*), it was determined that the *Dehalococcoides spp.* present contain the necessary RDase genes to fully dechlorinate TCE. The field measurements indicate that the current geochemical conditions (e.g. Fe^{2+}concentration, pH, sulfate concentration) and the slowly increasing ethene concentration inside the biowall can support (and are supporting) this important dechlorinating microbe albeit at low concentrations. The cell density needed to effectively combat the current contaminant mixture has not been determined at this time. Heavner et al. (2018) kept a relatively steady cell density of KB-1 *Dehalococcoides mccartyi* in continuously TCE-fed anaerobic batch reactors (approximately $1x10^{8}$ cells/mL) over the 24 h experiment and calculated a TCE respiration rate range of $9x10^{-13}$ to $2.88x10^{-12}$ µmol/cell·h (Heavner et al. 2018; Rowe et al. 2012).

Despite the low biomass extracted from the soil/biowall materials (<10^{7} bacteria/g material), the diverse microbial population identified with Illumina indicated that the microbial community currently present at the site provides a reasonably diverse inoculum with which to jump start a series of bioaugmentation experiments (Davis et al. 2002). The most robust collection of microorganisms would likely come from a combination of MW6 (demonstrated to contain *Dehalococcoides* with *bvcA*) and BW8 (largest fraction of *Desulfovibrionaceae*) that have demonstrated genetic diversity and active dechlorination (Wiedemeier et al. 1996; Ritalahti et al. 2006).

As an open system, the biowall will eventually degrade and/or have its degradation capacity reduced, requiring the replenishment of the fill-material. ZVI may also be introduced into the biowall to boost the environment's reducing conditions and degradation capability. ZVI shavings did not significantly affect the SDC-9 *Dehalococcoides* population inside the reactors used to model the interaction of these two treatment methods. Likewise, the groundwater-fed reactors had a similar *Dehalococcoides* population as those fed with the more nutrient-rich RAMM-media possibly indicating that biostimulation may not be necessary to support an actively dechlorinating *Dehalococcoides* population. These groundwater-fed reactors with and without ZVI also had a higher *bvcA* and *vcrA* content suggesting that a bioaugmented biowall (with or without ZVI shavings) could encourage a *Dehalococcoides* population targeted towards VC reduction. With the VC concentration measured in the field at levels higher than its MCL, this finding is very valuable in order to fully meet the remediation objectives outlined by the Superfund program.

The applicability of bioaugmentation and biostimulation efforts needs to be carefully evaluated prior to full scale implementation to avoid directly starving the targeted community or indirectly supporting communities able to outcompete it. Also, it is critical is to determine the lower population threshold needed to address the contamination level, and the manner in which to increase the population of a complex community that does not necessarily favor culturing. A system the requires continuous intervention is expensive and time consuming, which is why it is important to seek out approaches that will both minimize invasiveness and maximize TCE degradation.

Supplementary Material

Table 2-4 (Supplementary Table 1): Organism reference list

Kingdom	Phylum	Class	Order	Family	Key	Source
Archaea	*Euryarchaeota*	*Archaeoglobi*	*Archaeoglobales*	*Archaeoglobaceae*	SR	Davis et al. 2002; Bradley and Chapelle 1998
Archaea	*Euryarchaeota*	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*	M	Garcia 1990
Archaea	*Euryarchaeota*	*Methanobacteria*	*Methanobacteriales*	*Methanothermaceae*	M	Garcia 1990
Archaea	*Euryarchaeota*	*Methanococci*	*Methanococcales*	*Methanococcaceae*	M	Garcia 1990
Archaea	*Euryarchaeota*	*Methanomicrobia*	*Methanomicrobiales*	*Methanocorpusculaceae*	M	Garcia 1990
Archaea	*Euryarchaeota*	*Methanomicrobia*	*Methanomicrobiales*	*Methanomicrobiaceae*	M	Garcia 1990
Archaea	*Euryarchaeota*	*Methanomicrobia*	*Methanomicrobiales*	*Methanospirillaceae*	M	Dennis et al. 2003
Archaea	*Euryarchaeota*	*Methanomicrobia*	*Methanosarcinales*	*Methanosaetaceae*	M	Dennis et al. 2003
Archaea	*Euryarchaeota*	*Methanomicrobia*	*Methanosarcinales*	*Methanosarcinaceae*	M	Tandoi et al. 1994; Futagami et al. 2011, Garcia 1990
Bacteria	*Actinobacteria*	*Actinobacteria*	*Corynebacteriales*	*Mycobacteriaceae*		Gu et al. 2004; Freeborn et al. 2005
Bacteria	*Actinobacteria*	*Actinobacteria*	*Corynebacteriales*	*Nocardiaceae*	P, CoT	Gu et al. 2004; Semprini et al. 1997; Janssen et al. 2001
Bacteria	*Actinobacteria*	*Actinobacteria*	*Propionibacteriales*	*Propionibacteriaceae*	F	Gu et al. 2004; Zhang et al. 2015; Freeborn et al. 2005
Bacteria	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	F	Freeborn et al. 2005;

						Gu et al. 2004
Bacteria	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	F	Zhang et al. 2015
Bacteria	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*		Freeborn et al. 2005; Hemme et al. 2015
Bacteria	*Chloroflexi*	*Dehalococcoidia*	*Dehalococcoidales*	*Dehalococcoidaceae*	Cl	Gu et al. 2004; Cupples et al. 2004; Futagami et al. 2011; Sung et al. 2006
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*		Freeborn et al. 2005
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Carnobacteriaceae*	F	Zhang et al. 2015
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*		Ozturk et al. 2012; Freeborn et al. 2005
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	F	Zhang et al. 2015
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	A; Cl	Freeborn et al. 2005; Chang et al. 2000
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Eubacteriaceae*	Cl, A, F	Dennis et al. 2003; Futagami et al. 2011; Davis et al. 2002
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Peptococcaceae*	A, Cl, SR	Davis et al. 2002; Ozturk et al. 2012; Dugat-Bony et al. 2012; Bradley and Chapelle 1998
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Methylocystaceae*	Mt, CoT	Semprini et al. 1997; Fitch et al. 1996; Cupples et al. 2004
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Burkholderiaceae*	CoT, Ph,T	Hemme et al. 2015; Semprini et al. 1997
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*		Hemme et al. 2015
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Nitrosomonadales*	*Nitrosomonadaceae*	AO, CoT	Semprini et al. 1997
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*		Freeborn et al. 2005
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobacteraceae*	SR	Davis et al. 2002; Bradley and Chapelle 1998
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobulbaceae*	SR	Davis et al. 2002; Bradley and Chapelle 1998
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfovibrionales*	*Desulfomicrobiaceae*	SR	Davis et al. 2002; Bradley and Chapelle 1998

Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfovibrionales*	*Desulfovibrionaceae*	SR, F, OS	Zhang et al. 2015; Freeborn et al. 2005; Futagami et al 2011; Bradley and Chapelle 1998
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfuromonadales*	*Desulfuromonadaceae*	Cl	Smidt and de Vos 2004; Davis 2002
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	IR	Davis et al. 2002; Hemme et al. 2015; Smidt and de Vos 2004
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Myxococcales*	*Anaeromyxobacteraceae*		Smidt and de Vos 2004
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Syntrophobacterales*	*Syntrophaceae*	SR	Smidt et al. 2004; Holliger et al. 1993; Davis et al. 2002; Bradley and Chapelle 1998
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Syntrophobacterales*	*Syntrophobacteraceae*	OS	Zhang et al. 2015
Bacteria	*Proteobacteria*	*Epsilonproteobacteria*	*Campylobacterales*	*Campylobacteraceae*	Cl	Scholtz-Muramatsu et al. 1995: Smidt et al. 2004; Dugat-Bony et al. 2012
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Alteromonadales*	*Shewanellaceae*	IR	Dennis et al. 2003; Hemme et al. 2015
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacterales*	*Enterobacteriaceae*	F, Cl	Freeborn et al. 2005; Sharma and McCarty 1996
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Methylococcales*	*Methylococcaceae*	Mt	Hemme et al. 2015
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	DN	Hemme et al. 2015
Bacteria	*Synergistetes*	*Synergistia*	*Synergistales*	*Synergistaceae*	OS	Zhang et al. 2015; Dennis et al. 2003
Bacteria	*Thermodesulfobacteria*	*Thermodesulfobacteria*	*Thermodesulfobacteriales*	*Thermodesulfobacteriaceae*	SR	Davis et al. 2002; Bradley and Chapelle 1998
Bacteria				*Firmicutes*		Davis et al. 2002; Bradley and Chapelle 1998

Key (broad classifications): SR= sulfate reducing; F: fermentation; CoT= TCE co-metabolite; Cl= CAH metabolite; A= acetogen; OS= oxygen scavenger; IR= iron reducer; M= methanogen; Mt= methanotroph; AO= ammonia oxidizing; P= propane; DN= denitrifier; Ph= phenol; T= toluene

Table 2-5 (Supplementary Table 2): Description of the taxonomical hierarchy and frequencies for bacterial families in the soil and biowall samples

Family member	BW3 deep	BW3 shallow	BW6 deep	BW6 shallow	BW8	MW6
Acetobacteraceae	76	91	296	123	26	426
Acholeplasmataceae	112	115	371	191	66	27
Acidimicrobiaceae	24	51	111	51	22	31
Acidithiobacillaceae						3
Acidobacteriaceae	96	62	698	290	64	696
Actinomycetaceae	22	34	61	24	9	9
Actinopolysporaceae	21	36	70	42	23	
Aerococcaceae	10	3	14	2		5
Aeromonadaceae	2					148
Alcaligenaceae	14	24	29	12	9	35
Alcanivoracaceae		1	2			216
Alicyclobacillaceae	1	455	6	4	3	
Alteromonadaceae	19	12	29	11	8	579
Amoebophilaceae	7	34	38	25	8	405
Anaeroplasmataceae	3	1	41	11	2	1
Anaplasmataceae	14	33	95	25	11	9
Armatimonadaceae	13	42	61	42	8	1
Aurantimonadaceae	8	4	13	11	3	1
Bacillaceae	646	10344	1224	1579	378	798
Bacteriovoracaceae			1			
Bacteroidaceae	23	19	503	130	10	51
Balneolaceae		2				7
Bartonellaceae	17	35	52	22	27	3
Bdellovibrionaceae	11	26	20	7	12	1
Beijerinckiaceae	34	15	183	58	17	8
Bifidobacteriaceae	181	341	745	360	152	733
Bogoriellaceae	4	1	7	3		
Borreliaceae	12	18	32	5	9	25
Brachyspiraceae	11	19	24	12	7	1
Bradyrhizobiaceae	94	129	660	246	71	51
Brevibacteriaceae	1	1	2	3		2
Brucellaceae	3	5	4	6	3	5
Burkholderiaceae	105	183	264	80	66	298
Caldilineaceae	325	417	1145	514	204	54
Campylobacteraceae	11	27	103	39	7	11
Carnobacteriaceae	466	55	1417	517	197	3
Catenulisporaceae			3	1		
Caulobacteraceae	79	82	114	86	54	109
Cellulomonadaceae	45	54	415	134	18	154
Chitinophagaceae	334	511	817	233	196	319
Chlorobiaceae	14	29	82	24	17	1
Chromatiaceae	82	130	256	98	40	928
Chroococcaceae	5	7	8	2		21
Chrysiogenaceae	2	1	16	1	1	
Chthoniobacteraceae	44	99	662	304	74	782
Chthonomonadaceae	4	4	33	29		128
Clostridiaceae	3166	3503	12228	5983	2128	1175
Comamonadaceae	197	294	233	93	110	1639

Conexibacteraceae	24	41	91	42	21	
Coriobacteriaceae	75	56	237	114	26	16
Corynebacteriaceae	1	4	8	3	1	4
Coxiellaceae	74	110	292	141	28	29
Crenotrichaceae	8	1	25	17	1	
Cryomorphaceae	3	2	2		2	61
Cyanobacteriaceae			2		1	5
Deferribacteraceae	15	41	72	23	16	7
Dehalococcoidaceae	5	13	31	11	6	
Deinococcaceae	1183	1199	4102	1525	634	83
Dermabacteraceae	6	10	10	5	1	1
Dermacoccaceae			1			
Desulfobacteraceae	246	377	490	221	99	137
Desulfobulbaceae	17	4	16	13	9	125
Desulfohalobiaceae	120	86	269	130	49	4
Desulfomicrobiaceae	7	1	4			
Desulfovibrionaceae	202	306	576	257	167	202
Desulfurococcaceae		2				
Desulfuromonadaceae	26	26	106	44	22	259
Dietziaceae		2			1	2
Ectothiorhodospiraceae	116	154	270	150	73	904
Enterobacteriaceae	4	8	29	10	4	1097
Enterococcaceae	58	72	253	116	62	24
Entomoplasmataceae	1		10	4	3	2
Erysipelotrichaceae	224	58	946	308	33	
Erythrobacteraceae	54	89	109	55	32	101
Eubacteriaceae	3	12	20	8	3	5
Euzebyaceae	10	18	47	21	17	54
Ferrimonadaceae		3	1	6		
Fibrobacteraceae	1		4	2		
Fimbriimonadaceae	5	8	17		2	
Flammeovirgaceae	7	8	15	6	4	2
Flavobacteriaceae	714	707	2804	1295	417	577
Francisellaceae	1		4		3	3
Frankiaceae	4		14	2	3	
Fusobacteriaceae			1			92
Gallionellaceae	3	4	2		2	118
Gemmataceae	31	25	146	54	26	408
Gemmatimonadaceae	14	26	93	48	12	62
Geobacteraceae	463	476	1739	671	202	454
Geodermatophilaceae	2	1				
Glycomycetaceae	7	8	18	12	3	3
Gomphosphaeriaceae	4	4	13	3	4	60
Gordoniaceae	4		7		1	
Hahellaceae	1	3		1	1	
Halanaerobiaceae	3	4	11	3	3	
Halobacteriaceae	3	3		2		100
Halobacteroidaceae		5			2	1
Halomonadaceae	37	41	60	21	30	4181
Halothiobacillaceae		1	1			25
Helicobacteraceae	23	43	35	29	18	5
Heliobacteriaceae	65	114	156	49	28	12
Holophagaceae	77	82	457	150	36	85

Hydrogenophilaceae	11	21	28	10	6	86
Hyphomicrobiaceae	1020	1572	2541	975	662	650
Hyphomonadaceae	26	14	54	9	11	10
Iamiaceae	7	13	15	2	3	
Idiomarinaceae			3		1	11
Ignavibacteriaceae	539	678	788	425	213	113
Intrasporangiaceae	18	25	30	11	1	20
Isosphaeraceae	138	197	768	336	143	655
Kiloniellaceae	9	19	38	16	9	17
Kineosporiaceae	14	3	13	4	3	
Lachnospiraceae	63	94	457	312	121	24
Lactobacillaceae	9	45	34	16	13	3
Legionellaceae	33	32	115	42	23	199
Leptospiraceae	2	2	3	4	2	7
Leptotrichiaceae		4	11	4	3	1
Leuconostocaceae	250	334	982	462	214	251
Listeriaceae	7	12	16	5	2	7
Litoricolaceae	2	3	3	4		81
Methanobacteriaceae	19	25	87	42	10	1
Methanocellaceae	1	1	5	5	2	
Methanocorpusculaceae		1	1			
Methanomicrobiaceae	6	6	3	7	5	
Methanoregulaceae		4	6	5		
Methanosaetaceae	17	76	134	42	25	
Methanosarcinaceae	4	5	22	8	2	
Methanospirillaceae	8	3	7		2	
Methylacidiphilaceae	77	89	331	162	54	758
Methylobacteriaceae	18	31	72	22	11	53
Methylococcaceae	111	134	192	88	59	657
Methylocystaceae	230	283	712	301	151	51
Methylophilaceae	11	14	13	8	8	537
Microbacteriaceae	42	47	180	84	29	140
Micrococcaceae	31	202	63	33	24	15
Microcystaceae		1	2		2	1
Micromonosporaceae	97	159	206	105	58	160
Moraxellaceae	11	33	97	141	11	190
Moritellaceae	1		1	3	2	1
Mycobacteriaceae	34	44	84	39	12	72
Mycoplasmataceae	94	72	228	80	48	25
Myxococcaceae	81	69	422	164	26	6
Nannocystaceae	29	31	72	38	19	26
Neisseriaceae	47	46	142	39	16	262
Nitriliruptoraceae		1	1			
Nitrosomonadaceae	9	3		5	4	1
Nitrospinaceae				1		
Nitrospiraceae	4	6	11	7	3	228
Nocardiaceae	38	80	169	62	40	101
Nocardioidaceae	181	214	363	148	97	142
Nostocaceae	7	6	14	4	3	133
Oceanospirillaceae	22	29	49	29	28	1202
Odoribacteraceae		1	4	1		
Opitutaceae	47	44	104	48	22	107
Oscillochloridaceae		1	7			
Oxalobacteraceae	43	417	165	58	37	926

Paenibacillaceae	129	1246	354	220	89	163
Parachlamydiaceae	10	36	45	22	5	105
Pasteurellaceae		1	3	4	3	
Patulibacteraceae	1				1	
Peptococcaceae	208	228	497	220	115	314
Peptostreptococcaceae	611	507	2849	799	549	18
Phyllobacteriaceae	120	163	209	90	72	17
Piscirickettsiaceae	24	23	94	15	12	179
Planctomycetaceae	157	235	677	240	133	862
Planococcaceae	43	1253	143	270	26	349
Polyangiaceae	111	92	353	115	65	298
Porphyromonadaceae	285	250	827	380	126	2
Promicromonosporaceae		1	2	2		1
Propionibacteriaceae	9	1	25	16		
Pseudanabaenaceae	4	5	6	2	4	369
Pseudoalteromonadaceae	10	20	55	20	12	52
Pseudomonadaceae	63	219	63	32	31	460
Pseudonocardiaceae	161	244	634	308	166	460
Psychromonadaceae	1	1	3	4		2
Puniceicoccaceae	12	12	36	17	2	5
Rhabdochlamydiaceae	109	130	294	123	57	3
Rhizobiaceae	81	154	177	77	53	145
Rhodobacteraceae	59	83	112	59	42	303
Rhodobiaceae	18	27	28	19	15	1
Rhodocyclaceae	124	184	233	74	50	851
Rhodospirillaceae	392	483	1235	457	236	1138
Rhodothermaceae	16	23	84	35	4	3577
Rickettsiaceae	27	46	149	66	36	182
Rikenellaceae	3	4		2		
Rivulariaceae	1	2	4	6	1	771
Rubrobacteraceae	2	4	10	5	2	
Ruminococcaceae	22	38	102	50	28	57
Saccharospirillaceae	7	3	6		1	66
Sanguibacteraceae	1	2	8	2		4
Saprospiraceae	54	90	88	45	41	428
Shewanellaceae	15	11	35	13	8	889
Sinobacteraceae	194	282	381	180	126	233
Solibacteraceae	37	77	316	127	37	548
Solirubrobacteraceae	54	67	133	51	32	17
Sphingobacteriaceae	1317	1400	5294	1763	536	505
Sphingomonadaceae	715	925	1484	568	395	1104
Spirochaetaceae	214	234	516	229	77	57
Sporichthyaceae						1
Sporolactobacillaceae			1			
Staphylococcaceae	13	17	33	19	9	15
Streptococcaceae	9	21	37	21	5	9
Streptomycetaceae	28	65	118	59	28	206
Streptosporangiaceae	26	31	107	48	16	38
Symbiobacteriaceae	53	20	51	35	5	
Synechococcaceae			3			
Synergistaceae	12	16	69	27	9	2
Syntrophaceae	89	140	221	116	46	144
Syntrophobacteraceae	34	55	85	41	28	5
Syntrophomonadaceae	8	17	61	34	15	2

Thermaceae	10	10	33	12	10	4
Thermoactinomycetaceae	25	46	106	34	23	2
Thermoanaerobacteraceae	247	307	1033	416	155	288
Thermococcaceae	1					
Thermodesulfobacteriaceae	61	43	177	94	50	1
Thermogemmatisporaceae	142	163	510	219	105	277
Thermomonosporaceae	58	41	178	56	12	4
Thermoproteaceae	1	2	14	6	5	
Thermotogaceae	41	78	202	100	41	249
Thiotrichaceae	13	9	22	9	3	337
Tsukamurellaceae	7		2	6	1	
Veillonellaceae	259	371	955	471	240	274
Verrucomicrobiaceae	72	144	105	38	54	1033
Vibrionaceae		1	3	5		4
Waddliaceae	140	205	451	176	91	3
Xanthobacteraceae	162	225	461	201	114	150
Xanthomonadaceae	287	369	454	195	101	424
Grand Total (>0.1%)	**20292**	**36750**	**67709**	**29700**	**12348**	**44450**

Gray cells= below 0.1% cutoff value; Blank cells= not present

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Mention of specific products is for identification purposes only and does not imply endorsement by the US Department of Agriculture, the Federal Government, nor the University of Maryland to the exclusion of other suitable products or suppliers.

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References

[ATSDR] Agency for Toxic Substances and Disease Registry (2006) Toxic substances portal: Vinyl chloride. https://www.atsdr.cdc.gov/phs/phs.asp?id=280&tid=51 . Accessed 20 April 2018.

ATSDR (2011) Toxic substances portal: Trichloroethylene (TCE). https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=30 . Accessed 20 April 2018.

ATSDR (2015) Summary data for 2015 priority list of hazardous substances. https://www.atsdr.cdc.gov/spl/resources/atsdr_2015_spl_detailed_data_table.pdf . Accessed 20 April 2018.

ATSDR (2017) ATSDR's substance priority list. https://www.atsdr.cdc.gov/spl/ . Accessed 20 April 2018.

Atashgahi S, Lu Y, Zheng Y, Saccenti E, Suarez-Diez M, Ramiro-Garcia J, Eisenmann H, Elsner M, J.M. Stams A, Springael D, Dejonghe W, Smidt H (2017) Geochemical and microbial community determinants of reductive dechlorination at a site biostimulated with glycerol. Environ Microbiol 19:968–981 . doi: 10.1111/1462-2920.13531

Behrens S, Azizian MF, McMurdie PJ, Sabalowsky A, Dolan ME, Semprini L, Spormann AM (2008) Monitoring abundance and expression of "*Dehalococcoides*" species chloroethene-reductive dehalogenases in a tetrachloroethene-dechlorinating flow column. Appl Environ Microbiol 74:5695–5703 . doi: 10.1128/AEM.00926-08

BMT Entech, Inc. (2009) Proposed plan for the Beaver Dam Road landfill (BARC 27).

BMT Designers and Planners, Inc. (2015) Quarterly performance monitoring report, June through September 2015: Year 2, fourth quarter (Draft).

BMT Designers and Planners, Inc. (2016) Monitoring well installation work plan, Beaverdam road landfill (BARC 27). Task order no. AG-32SC-P-15-0376.

Bradley PM, Chapelle FH (1998) Microbial mineralization of VC and DCE under different terminal electron accepting conditions. Anaerobe 4:81-87

Chang YC, Hatsu M, Jung K, Yoo YS, Takamizawa K (2000) Isolation and characterization of a teterachloroethylene dechlorinationg bacterium *Clostridium bifermentans* DPH-1. J Biosci Bioeng 89:489-491

Chen J, Xiu Z, Lowry GV, Alvarez PJJ (2011) Effect of natural organic matter on toxicity and reactivity of nano-scale zero-valent iron. Water Res 45:1995–2001 . doi: 10.1016/j.watres.2010.11.036

Chiao FF, Currie RC, McKone TE (1994) Intermedia transfer factors for contaminants found at hazardous waste sites: Trichloroethylene (TCE). Prepared for the Office of Scientific Affairs, the Department of Toxic Substances Control (DTSC) and the California Environmental Protection Agency in Support of the CalTOX Model.

Cupples AM, Spormann AM, McCarty PL (2004) Comparative evaluation of chloroethene dechlorination to ethene by *Dehalococcoides*-like

microorganisms. Environ Sci Technol 38:4768–4774 . doi: 10.1021/es049965z
Da Silva ML, Alvarez PJ (2008) Exploring the correlation between halorespirer biomarker concentrations and TCE dechlorination rates. J Environ Eng 134:895–901 . doi: 10.1061/(ASCE)0733-9372(2008)134:11(895)
Davis JW, Odom JM, DeWeerd KA, Stahl DA, Fishbain SS, West RJ, Klecka GM, DeCarolis JG (2002) Natural attenuation of chlorinated solvents at Area 6, Dover Air Force Base: Characterization of microbial community structure. J Contam Hydrol 57:41–59
Dennis PC, Sleep BE, Fulthorpe RR, Liss SN (2003) Phylogenetic analysis of bacterial populations in an anerobic microbial consortium capable of degrading saturation concentrations of tetrachloroethylene. Can J Microbiol 49:15-27 . doi: 10.1139/W03-008
Diao M, Yao M (2009) Use of zero-valent iron nanoparticles in inactivating microbes. Water Res 43:5243–5251 . doi: 10.1016/j.watres.2009.08.051
Dong Y, Liang X, Krumholz LR, Philp RP, Butler EC (2009) The relative contributions of abiotic and microbial processes to the transformation of tetrachloroethylene and trichloroethylene in anaerobic microcosms. Environ Sci Technol 43:690–697 . doi: 10.1021/es801917p
Dugat-Bony E, Biderre-Petit C, Jaziri F, David MM, Denonfoux J, Lyon DY, Richard JY, Curvers C, Boucher D, Vogel TM, Peyretaillade E, Peyret P (2012) In situ TCE degradation mediated by complex dehalorespiring communities during biostimulation processes. Microb Biotechnol 5:642–653 . doi: 10.1111/j.1751-7915.2012.00339.x
Ellis DE, Lutz EJ, Odom JM, Buchanan RJ, Bartlett CL, Lee MD, Harkness MR, Deweerd KA (2000) Bioaugmentation for accelerated in situ anaerobic bioremediation. Environ Sci Technol 34:2254–2260 . doi: 10.1021/es990638e
Farrell J, Kason M, Melitas N, Li T (2000) Investigation of the long-term performance of zero-valent iron for reductive dechlorination of trichloroethylene. Environ Sci Technol 34:514–521 .
Fitch MW, Speitel GE, Georgiou G (1996) Degradation of trichloroethylene by methanol-grown cultures of Methylosinus trichosporium OB3b PP358. Appl Environ Microbiol 62:1124
Freeborn RA, West KA, Bhupathiraju VK, Chauhan S, Rahm BG, Richardson RE, Alvarez-Cohen L (2005) Phylogenetic analysis of TCE-dechlorinating consortia enriched on a variety of electron donors. Environ Sci Technol doi: 10.1021/es048003p
Fung JM, Morris RM, Adrian L, Zinder SH (2007) Expression of reductive dehalogenase genes in *Dehalococcoides ethenogenes* strain 195 growing on tetrachloroethene, trichloroethene, or 2,3-dichlorophenol. Appl Environ Microbiol 73:4439–4445 . doi: 10.1128/AEM.00215-07
Futagami T, Okamoto F, Hashimoto H, Fukuzawa K, Higashi K, Nazmul Hussain Nazir KHM, Wada E, Suyama A, Takegawa K, Goto M, Nakamura K, Furukawa K (2011) Enrichment and characterization of a trichloroethene-dechlorinating consortium containing multiple "*Dehalococcoides*" strains. Biosci Biotechnol Biochem 75:1268-1274

Garcia JL (1990) Taxnomy and ecology of methanogens. FEMS Microbiol Lett 87:297-308 . doi: 10.1016/0378-1097(90)90470-B

GraphPad Prism (2017) Interpreting results: Kruskal-Wallis test. http://www.graphpad.com/guides/prism/7/statistics/index.htm?how_the_kruskal-wallis_test_works.htm . Accessed 20 April 2018.

Gu B, Phelps TJ, Liang L, Dickey MJ, Roh Y, Kinsall BL, Palumbo AV, Jacobs GK (1999) Biogeochemical dynamics in zero-valent iron columns: Implication for permeable reactive barriers. Environ Sci Technol 33:2170–2177

Gu AZ, Hedlund BP, Staley JT, Strand SE, Stensel HD (2004) Analysis and comparison of the microbial community structures of two enrichment cultures capable of reductively dechlorinating TCE and cis-DCE. Environ Microbiol doi: 10.1046/j.1462-2920.2003.00525.x

Haest PJ, Springael D, Smolders E (2010) Dechlorination kinetics of TCE at toxic TCE concentrations: Assessment of different models. Water Res 44:331–339 . doi: 10.1016/j.watres.2009.09.033

Harkness MR, Bracco AA, Brennan MJ, DeWeerd KA, Spivack JL (1999) Use of bioaugmentation to stimulate complete reductive dechlorination of trichloroethene in Dover soil columns. Environ Sci Technol 33:1100-1109 . doi: 10.1021/es9807690

He J, Ritalahti K, Aiello M, Loffler F (2003) Complete detoxification of vinyl chloride by an anerobic enrichment culture and identification of the reductivity dechlorinating population as a *Dehalococcoides* species. Appl Env Microb 69:996–1003 . doi: 10.1128/AEM.69.2.996

He J, Sung Y, Krajmalnik-Brown R, Ritalahti KM, Löffler FE (2005) Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. Environ Microbiol 7:1442–1450 . doi: 10.1111/j.1462-2920.2005.00830.x

Heavner GLW, Mansfeldt CB, Debs GE, Hellerstedt ST, Rowe AR, Richardson RE (2018) Biomarkers' responses to reductive dechlorination rates and oxygen stress in bioaugmentation culture KB-1TM. Microorganisms 6:pii: E13 . doi: 10.3390/microorganisms6010013

Hemme CL, Tu Q, Shi Z, Qin Y, Gao W, Ddeng Y, Van Nostrand JD, Wu L, He Z, Chain PSG, Tringe SG, Fields MW, Rubin EM, Tiedje JM, Hazen TC, Arkin AP, Zhou J (2015) Comparative metagenomics reveals impact of contaminants on groundwater microbiomes. Front Microbiol doi: 10.3389/fmicb.2015.01205

Hendrickson ER, Payne JA, Young RM, Starr MG, Perry MP, Fahnestock S, Ellis DE, Ebersole RC (2002) Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. Appl Environ Microbiol 68:485–95 . doi: 10.1128/AEM.68.2.485-495.2002

Holliger C, Schraa G, Stams AJM, Zehnder AJB (1993) A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Appl Environ Microbiol 59:2991–2997

Holmes, V.F., He, J., Lee, P.K.H., Alvarez-Cohen, L., 2006. Discrimination of multiple *Dehalococcoides* strains in a trichloroethene enrichment by quantification of their reductive dehalogenase genes. Appl. Environ. Microb. 72, 5877-5883. https://doi.org/10.1128/AEM.00516-06

Hood ED, Major DW, Quinn JW, Yoon W-S, Gavaskar A, Edwards EA (2008) Demonstration of enhanced bioremediation in a TCE source area at Launch Complex 34, Cape Canaveral Air Force Station. Groundw Monit Remediat 28:98–107 . doi: 10.1111/j.1745-6592.2008.00197.x

Ingram DT, Callahan MT, Ferguson S, Hoover DG, Chiu PC, Shelton DR, Millner PD, Camp MJ, Patel JR, Kniel KE, Sharma M (2012) Use of zero-valent iron biosand filters to reduce *Escherichia coli* O157:H12 in irrigation water applied to spinach plants in a field setting. J Appl Microbiol 112(3):551–560. https://doi.org/10.1111/ j.1365-2672.2011.05217.x

Jacoby WA, Blake DM, Watt AS (1998) Remediation of trichloroethylene or perchloroethylene contamination. In: Meyers RA (ed) Encyclopedia Weries in Environmental Sciences: Environmental Analysis and Remediation, Wiley-Blackwell, New Jersey, pp 4847-4873

Janssen DB, Oppentocht JE, Poelarends GJ (2001) Microbial dehalogenation. Curr Opin Biotechnol 12:254-258

Johnson DR, Lee PKH, Holmes VF, Fortin AC, Alvarez-Cohen L (2005) Transcriptional expression of the *tceA* gene in a *Dehalococcoides*-containing microbial enrichment. Appl Environ Microbiol 71:7145–7151 . doi: 10.1128/AEM.71.11.7145

Krajmalnik-Brown R, Holscher T, Thomson IN, Saunders FM, Ritalahti KM, Loffler FE (2004) Genetic identification of a putative vinyl chloride reductase in strain BAV1. Appl Environ Microbiol 70:6347–6351 . doi: 10.1128/AEM.70.10.6347

Lee JH, Dolan M, Field J, Istok J (2010) Monitoring bioaugmentation with single-well push-pull tests in sediment systems contaminated with trichloroethene. Environ Sci Technol 44:1085-1092

Lee PKH, Johnson DR, Holmes VF, He J, Alvarez-Cohen L (2006) Reductive dehalogenase gene expression as a biomarker for physiological activity of *Dehalococcoides* spp. Appl Environ Microbiol 72:6161–6168 . doi: 10.1128/AEM.01070-06

Lee PKH, Macbeth TW, Sorenson KS, Deeb RA, Alvarez-Cohen L (2008) Quantifying genes and transcripts to assess the in situ physiology of "*Dehalococcoides*" spp. in a trichloroethene-contaminated groundwater site. Appl Environ Microbiol 74:2728–2739 . doi: 10.1128/AEM.02199-07

Lee PKH, Warnecke F, Brodie EL, Macbeth TW, Conrad ME, Andersen GL, Alvarez-Cohen L (2012) Phylogenetic microarray analysis of a microbial community performing reductive dechlorination at a TCE-contaminated site. Environ Sci Technol 46:1044–1054 . doi: 10.1021/es203005k

Lendvay JM, Löffler FE, Dollhopf M, Aiello MR, Daniels G, Fathepure BZ, Gebhard M, Heine R, Helton R, Shi J, Krajmalnik-Brown R, Major CL, Barcelona MJ, Petrovskis E, Hickey R, Tiedje JM, Adriaens P (2003) Bioreactive barriers: A comparison of bioaugmentation and biostimulation for chlorinated solvent remediation. Environ Sci Technol 37:1422-1431 . doi: 10.1021/ES025985U

Löffler FE, Sun Q, Li J, Tiedje JM (2000) 16S rRNA gene-based detection of tetrachloroethene-dechlorinating *Desulfuromonas* and *Dehalococcoides* species. Appl Environ Microbiol 66:1369

Löffler FE, Yan J, Ritalahti KM, Adrian L, Edwards EA, Konstantinidis KT, Müller JA, Fullerton H, Zinder SH, Spormann AM (2013) *Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, *Dehalococcoidia* classis nov., order *Dehalococcoidales* ord. nov. and famil. Int J Syst Evol Microbiol 63:625–635 . doi: 10.1099/ijs.0.034926-0

Major DW, McMaster ML, Cox EE, Edwards EA, Dworatzek SM, Hendrickson ER, Starr MG, Payne JA, Buonamici LW (2002) Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. Environ Sci Technol 36:5106–16

Maymó-Gatell X, Chien YT, Gossett JM, Zinder SH (1997) Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 276:1568–1571 . doi: 10.1126/science.276.5318.1568

McDonald F, Tribes T, Baker-Kircher D, Back CM, Jacob C, Venot C (2012) Treatability testing : Biostimulation and bioaugmentation of a TCE groundwater plume on tribal land. US EPA Technology News and Trends 62:1–10

McDonald JH (2014) Handbook of biological statistics, 3rd edn. Sparky House Publishing, Baltimore, Maryland

Müller JA, Rosner BM, von Abendroth G, Meshulam-Simon G, McCarty PL, Spormann AM (2004) Molecular identification of the catabolic vinyl chloride reductase from *Dehalococcoides* sp. Strain VS and its environmental distribution. Appl Environ Microbiol 70:4880-4888

Niño de Guzmán GT, Hapeman CJ, Millner PD, McConnell LL, Michaelson DE, Jackson D, Kindig D, Kjellerup BV, Torrents A (2018a) Using adaptive management to guide multiple partners in TCE remediation using a permeable reactive barrier. In review Environ Manage

Niño de Guzmán GT, Hapeman CJ, Millner PD, McConnell LL, Jackson D, Kindig D, Torrents A (2018b) Using a high-organic matter biowall to treat a trichloroethylene plume at the Beaver Dam Road landfill. Environ Sci Pollut R 25:8735-8746 . doi: 10.1007/s11356-017-1137-1

Nishimura M, Ebisawa M, Sakihara S, Kobayashi A, Nakama T, Okochi M, Yohda M (2008) Detection and identification of Dehalococcoides species responsible for in situdechlorination of trichloroethene to ethene enhanced by hydrogen-releasing compounds. Biotechnol Appl Biochem doi: 10.1042/BA20070171

Orth WS, Gillham RW (1996) Dechlorination of trichloroethene in aqueous solution using Fe 0. Environ Sci Technol 30:66–71 . doi: 10.1021/es950053u

Ozturk Z, Tansel B, Katsenovich Y, Sukop M, Laha S (2012) Highly organic natural media as permeable reactive barriers: TCE partitioning and anaerobic degradation profile in eucalyptus mulch and compost. Chemosphere 89:665-671

Popat SC, Deshusses MA (2010) Analysis of the rate-limiting step of an anaerobic biotrickling filter removing TCE vapors. Process Biochem 45:549–555 . doi: 10.1016/j.procbio.2009.11.017

Powell RM, Blowes DW, Gillham RW, Schultz D, Sivavec T, Puls RW, Vogan JL, Powell PD, Landis R (1998) Permeable reactive barrier technologies for contaminant remediation. EPA/600/R-98/125

Rahm BG, Chauhan S, Holmes VF, Macbeth TW, Jr. Sorenson KS, Alvarez-Cohen L (2006) Molecular characterization of microbial populations at two sites with differing reductive dechlorination abilities. Biodegradation 17:523–534 . doi: 10.1007/s10532-005-9023-9

Ritalahti KM, Amos BK, Sung Y, Wu Q, Koenigsberg SS, Löffler FE (2006) Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. Appl Environ Microbiol 72:2765–2774 . doi: 10.1128/AEM.72.4.2765-2774.2006

Rowe AR, Heavner GL, Mansfeldt CB, Werner JJ, Richardson RE (2012) Relating chloroethene respiration rates in *Dehalococcoides* to protein and mRNA biomarkers. Environ Sci Technol 46:9388–9397 . doi: 10.1021/es300996c

Semprini L (1997) Strategies for the aerobic co-matabolism of chlorinated solvents. Curr Opin Biotechnol 8:296–308

Seshadri R, Adrian L, Fouts DE, Eisen JA, Phillippy AM, Methe BA, Ward NL, Nelson WC, Deboy RT, Khouri HM, Kolonay JF, Dodson RJ, Daugherty SC, Brinkac LM, Sullivan SA, Madupu R, Nelson KE, Kang KH, Impraim M, Tran K, Robinson JM, Forberger HA, Fraser CM, Zinder SH, Heidelberg JF (2005) Genome sequence of the PCE-dechlorinating bacterium *Dehalococcoides ethenogenes*. Science 307:105–108 . doi: 10.1126/science.1102226

Scholtz-Muramatsu H, Neumann A, Meβmer M, Moore E, Diekert G (1995) Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium. Arch Microbiol 163:48-56

Sharma PK, McCarty PL (1996) Isolation and characterization of a facultatively aerobic bacterium that reductively dehalogenates tetrachloroethene to cis-1,2-Dichloroethene. Appl Environ Microbiol 62:761-765

Shelton DR, Tiedje JM (1984) Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl Environ Microbiol 48:840–848

US EPA (2009) National primary drinking water regulations. U.S. Environmental Protection Agency, EPA/816/F/09/004. https://www.epa.gov/sites/production/files/2016-06/documents/npwdr_complete_table.pdf. Accessed 12 May 2017

US EPA (2016) Trichloroethylene (tce): Chemistry and behavior. https://clu-in.org/contaminantfocus/default.focus/sec/trichloroethylene_(tce)/cat/Chemistry_and_Behavior/. Accessed 13 April 2017.

Vainberg S, Condee CW, Steffan RJ (2009) Large-scale production of bacterial consortia for remediation of chlorinated solvent-contaminated groundwater. J Ind Microbiol Biotechnol 36:1189-1197.

van der Zaan B, Hannes F, Hoekstra N, Rijnaarts H, De Vos WM, Smidt H, Gerritse J (2010) Correlation of *Dehalococcoides* 16S rRNA and chloroethene-reductive dehalogenase genes with geochemical conditions in chloroethene-contaminated groundwater. Appl Environ Microbiol 76:843–850 . doi: 10.1128/AEM.01482-09

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697

Wiedemeier TH, Newell CJ, Rifai HS, Wilson JT (1996) Natural attenuation of fuels and chlorinated solvents in the subsurface. John Wiley & Sons, New York, pp 617. https://doi.org/10.1002/9780470172964

Wilkin RT, Puls RW, Sewell GW (2003) Long-term performance of permeable reactive barriers using zero-valent iron: Geochemical and microbiological effects. Ground Water 41:493-503

Wolfe RS (2011) Techniques for cultivating methanogens. doi: 10.1016/B978-0-12-385112-3.00001-9

Yan J, Şimşir B, Farmer AT, Bi M, Yang Y, Campagna SR, Löffler FE (2016) The corrinoid cofactor of reductive dehalogenases affects dechlorination rates and extents in organohalide-respiring *Dehalococcoides mccartyi*. ISME J 10:1092–1101 . doi: 10.1038/ismej.2015.197

Yan J, Rash BA, Rainey FA, Moe WM (2009) Detection and quantification of *Dehalogenimonas* and "*Dehalococcoides*" populations via PCR-based protocols targeting 16S rRNA genes. Appl Environ Microbiol 75:7560-7564

Yi S, Seth EC, Men Y-J, Stabler SP, Allen RH, Alvarez-Cohen L, Taga ME (2012) Versatility in Corrinoid Salvaging and Remodeling Pathways Supports Corrinoid-Dependent Metabolism in Dehalococcoides mccartyi. Appl Environ Microbiol 78:7745–7752 . doi: 10.1128/AEM.02150-12

Zhang T, Shao M-F, Ye L (2012) 454 Pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. ISME J 6:1137–1147 . doi: 10.1038/ismej.2011.188

Zhang Y, Hu M, Li P, Wang X, Meng Q (2015) Analysis of trichloroethylene removal and bacterial community function based on pH-adjusted in an upflow anaerobic sludge blanket reactor. Appl Microbiol Biotechnol 99:9289-9297

Chapter 4: Using adaptive management to guide multiple partners in TCE remediation using a permeable reactive barrier

The findings of this chapter have been submitted to the *Journal of Environmental Management* and are in review.

Abstract

The US Department of Agriculture-Agricultural Research Service (USDA-ARS) worked together with the University of Maryland, College Park and BMT Designers and Planners (Consultant) to design a biowall to remediate the groundwater of a Superfund site located in Beltsville, MD. The U.S. Environmental Protection Agency (US EPA) oversaw the remediation plan as per the regulations of the Superfund program. But, a strategy was needed to guarantee the use of a science-based approach to the remediation efforts and to ensure that new information could be incorporated into the remediation plan. Thus, adaptive management was utilized by all three organizations to avoid the shortcomings of other remediation efforts elsewhere. Laboratory experiments and a historic-data assessment were conducted in conjunction with the monitoring plan to provide the Consultant and USDA with comprehensive feedback, to strengthen and to modify the monitoring and biowall construction plans as the requirements of the site changed. This feedback mechanism was repeated multiple times to make certain that the highest quality and most effective methods were used. The scope of the project also grew to include investigations of the soil microbial community for future structural biostimulation and bioaugmentation

activities. The biowall is currently functioning as planned, and the concentration of trichloroethylene (TCE) has been reduced downstream of the structure to levels at or below its Maximum Contaminant Level.

Introduction

The Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), also known as Superfund, was enacted by the US Congress in response to several environmental and human health disasters that had taken place during the previous decade (US EPA, 2013). The Act follows the criteria of the Hazard Ranking System (HRS), a checklist which assess the relative potential threat of a site to public health and the environment, and a comments and response period to compile the National Priorities List (NPL) (US EPA, 2011, 2012C). Sites can be discovered and identified by anyone, including citizens, state agencies, the United States Environmental Protection Agency (US EPA), and the responsible party. The US EPA and/or the responsible party are then required to address the issue.

The site in this project is a landfill that occupies approximately 1 hectare at the US Department of Agriculture-Agricultural Research Service (USDA-ARS) Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland, USA. BARC was included on the NPL in 1994, and the landfill was preliminarily identified as one of several Areas of Concern (AoCs) due to its history. The landfill was used for disposal of construction debris and demolition rubble from the 1940's to the 1980's and was capped in 1990 adhering to existing state regulations governing such facilities (BMT

Entech Inc., 2008, 2009; USDA-ARS, 2012). In 1998, it was formally labeled an AoC after trichloroethylene (TCE) was identified in the groundwater system surrounding the landfill at a concentration several orders of magnitude above the Maximum Contaminant Level (MCL) of 5 ppb (0.005 mg/L), posing a potential threat to human and environmental health (USDA-ARS, 2012; US EPA, 2009B).

The degradation products of TCE are also of great concern. The MCLs for 1,1-dichloroethylene, *cis*-/*trans*-1,2-dichloroethylene, and vinyl chloride are 0.007 mg/L, 0.07 mg/L, 0.1 mg/L, and 0.002 mg/L, respectively (ATSDR, 2006; US EPA, 1995). Though no MCL for ethane exists, it is highly flammable (flashpoint = -136.6°C) (Airgas 2004). The TCE plume, which is southeast of the landfill, has been estimated to be approximately 200 m long with a maximum width of approximately 140 m (BMT Entech Inc., 2009). The surrounding area includes a wetland and an unnamed branch of Beaver Dam Creek south of the landfill.

As part of the CERCLA process, a federally-led feasibility study was carried out in the mid to late 2000's to characterize the site, after which several action alternatives for remediation were proposed. The preferred remedy, construction of a mulch biowall, was chosen based on the study results (BMT Entech Inc., 2009; USDA-ARS, 2012; US EPA, 2009A), and this decision was documented in the Record of Decision (ROD) (BMT Designers & Planners, 2011). An academic institution (University of Maryland, College Park) was chosen to assist in implementation of the Record of

Decision. This collaboration also provided student training opportunities in a real-life setting.

This paper examines the iterative management and science implementation of this trilateral collaboration to mitigate TCE and its degradation products effectively. Adaptive management is a powerful though sometimes misused tool for evaluating problems and decisions that have a high degree of complexity and uncertainty (Gregory et al., 2006). Passive adaptive management relies on historical data and information to predict future site behavior followed by development of an action plan (Gregory et al., 2006). During the process, new information (as it becomes available) is used to update data sets, hypotheses, and action plan(s) as needed. This routine is similar to the method followed by CERCLA to inform the selection of an action remedy. Alternatively, active adaptive management relies on experimentation to provide relevant new information in order to determine the best course of action; in other words, several planned alternatives are tested in series or parallel, the results are compared, and then action is taken (Gregory et al., 2006). This process is more familiar to a laboratory setting.

In this project, a hybrid form of adaptive management was utilized to accommodate the short- and long-term needs and missions of the partners involved and to address the reigning objectives of the project. The first objective was to avoid the shortcomings of previous remediation efforts utilizing biowalls at other sites to inform the construction and monitoring of the biowall, and the second was to decrease the contaminant groundwater concentrations to meet the required federal standards (FRTR, 2003; Gilbert et al., 2013; Groundwater Services, Inc., 2004; Lu et al., 2008; Phillips et al., 2010; Vogan et al., 1999; Wilkin et al., 2005).

Methods

Assessment of historical data

USDA, the academic partner, and the consultant reviewed all previous investigative studies and all historical analytical site data related to the landfill, surrounding area, and aquifer analysis, as well as the basis for the biowall design and the preliminary monitoring plan. The focal criteria and parameters were to identify the data necessary to carry out the modeling of the contaminant fate and transport; to identify the samples required to ensure appropriate data are acquired for the modeling efforts; to ensure that the sampling locations are appropriate to gather the necessary information to track contaminant movement, degradation, and site evolution; to determine how many samples are sufficient for data quality requirements to demonstrate degradation efficacy; and to formulate a suitable monitoring schedule.

Laboratory experiments

Before construction, soil and water samples were collected from the uncontaminated portion of the study site and were used to assemble background control batch and flow-through reactors. Experiments were conducted concurrent to the historical data review to examine the physical and chemical properties of the wall components and to test the degradation and sorption capacity of a series of biowall fill materials (Niño de Guzmán et al., 2018a). Parameters measured included carbon content, porosity, redox conditions, and pH. Batch reactors containing test materials, such as mulch, compost, zero-valent iron (ZVI), and glycerol, were spiked with a TCE concentration similar to that found at the study site and were kept at the oxygen concentration and temperature of the groundwater at the landfill. Flow-through reactors were assembled consisting of the material mixtures demonstrating the greatest conversion of TCE to ethane in the batch reactors. Finally, the native soil microbial community was examined to determine if any anaerobic bacteria or communities demonstrating TCE degradation potential could be isolated and purposely introduced into the biowall materials (Niño de Guzmán et al., 2018b).

Field and biowall measurements

In accordance with the ROD, an approved groundwater monitoring program was assembled by the consultant. The sampling collection program designed by the consultant consisted of biweekly (liquid) samples from the biowall wells and biannual (liquid) samples from the biowall, transect wells, original site investigation groundwater monitoring wells, and Beaver Dam Creek. Physical parameters and inorganic and organic compounds were also measured in biowall well samples (Table 3-1) (Niño de Guzmán et al., 2018a; BMT 2014).

Table 3-1: Summary of monitoring analyses

Wells	Analyses		
	Physical Parameters	Inorganic Compounds	Organic Compounds
Biowall Wells (BW)	Dissolved Oxygen Redox Potential Temperature pH Salinity Turbidity Specific Conductivity	$CaCO_3$ Alkalinity Total Ferrous Iron Dissolved Ferrous Iron	VOCs Methane Ethane Ethene Total Organic Carbon
Transect Wells (TW)	Dissolved Oxygen Redox Potential Temperature pH Salinity Turbidity Specific Conductivity	None	VOCs
Remedial Investigation Wells (MW)	Dissolved Oxygen Temperature	None	VOCs
Surface Water	Dissolved Oxygen Redox Potential Temperature pH Salinity Turbidity Specific Conductivity	None	VOCs

Additional soil and water samples were collected from locations identified as optimal to measure the concentration and migration of TCE and degradation products. The sampling locations up-gradient and down-gradient of the biowall are shown in Figure

3-1. One year after the biowall was installed, samples were collected from within the structure to examine microbial migration, degradation activity, and whether contaminant equilibrium had been reached.

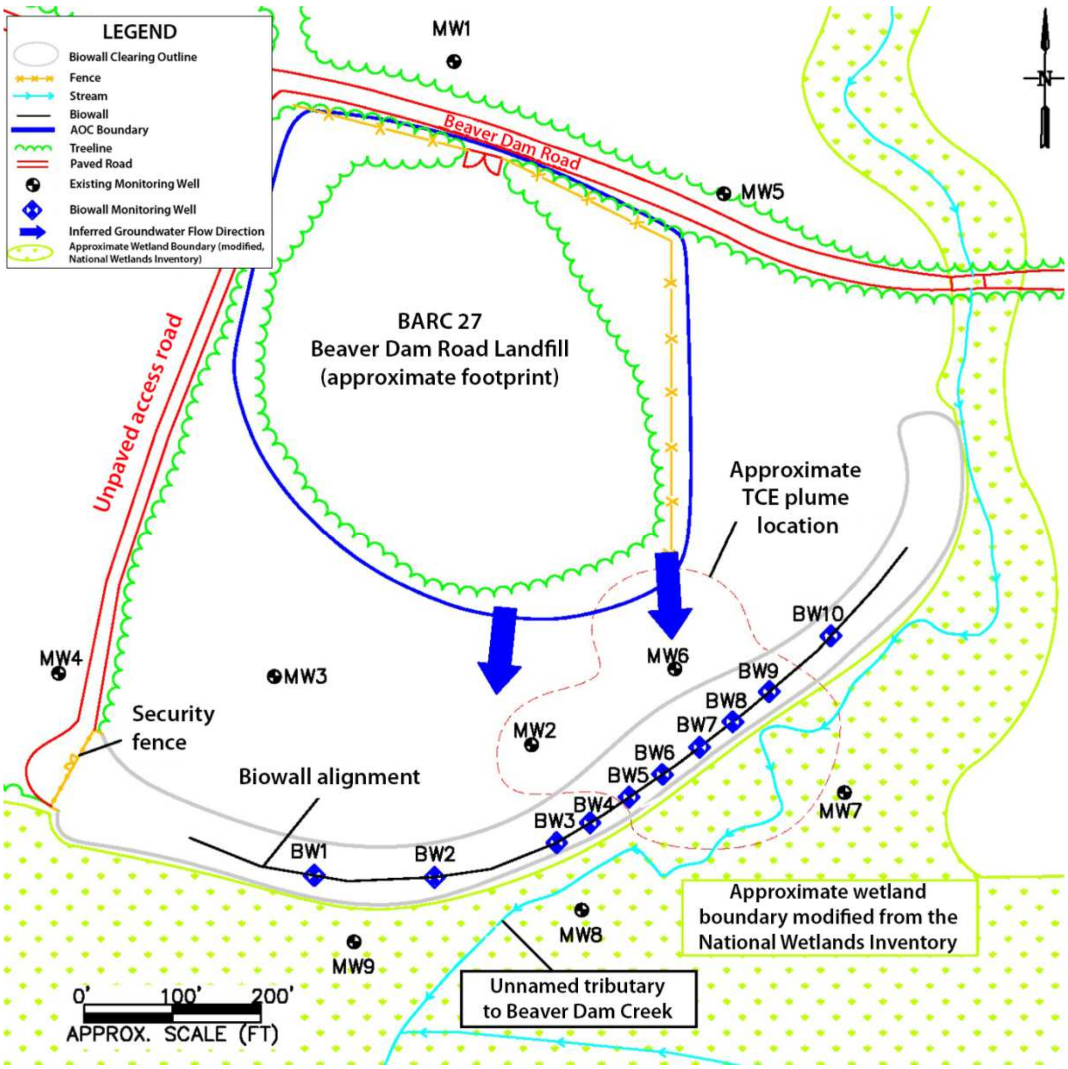

Figure 3-1: Site map with the location of the biowall structure, the biowall wells (BW), and the Remedial Investigation (MW) wells, marked. Modified from BMT's (the consultant) "BARC 27: Beaverdam Road Landfill Biowall Location Map".

Meetings and communication

Beginning in 2012, quarterly meetings have been held to facilitate communication between US EPA, USDA, the academic partner, and the consultant. These meetings were a forum to provide updates that generally included field monitoring test results, current research activities and preliminary results, future planned experiments, and plans for the following quarter. Frank discussions helped to avoid duplications of effort, scheduling conflicts, and miscommunication. Figure 3-2 illustrates the remediation project hierarchy and the most-commonly followed communication tree. More regular communication took place throughout the quarter between the USDA, the academic partner, and the consultant in the form of emails, phone calls, and site visits. The US EPA was not typically involved on a day-to-day basis and served as an overseer to ensure that the overall process was moving forward.

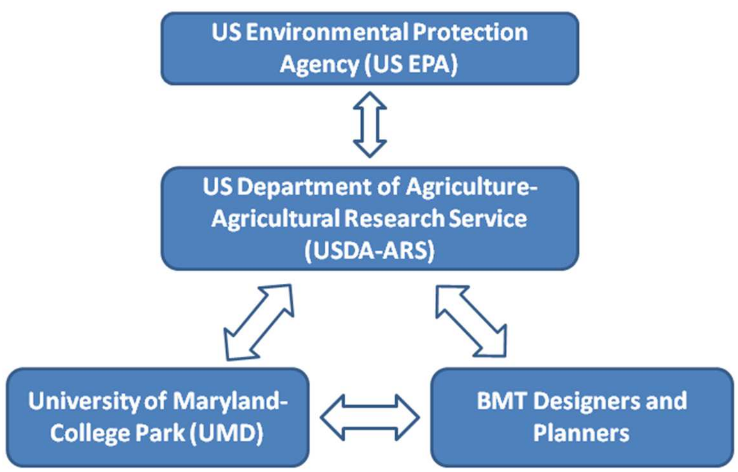

Figure 3-2: Communication pathways between institutions involved in the Beaverdam Road Landfill remediation project.

Reports

The consultant prepared and distributed draft quarterly monitoring reports summarizing the monitoring activities, sample test results, current biowall conditions, and general recommendations made during the last meeting. These documents were distributed at least two weeks before the quarterly meeting to give all the parties a chance to comment and to critique the materials. Yearly reports were compiled from these quarterly documents and formally submitted to the US EPA.

Results and discussion

Assessment of historical data

The first available groundwater and soils datasets came from a 1997 baseline groundwater sampling operation conducted by the consulting engineers. Their datasets were used to assess groundwater quality, flow patterns, and hydraulic characteristics (ENTECH, Inc., 1998). A 1998 site screening study provided a second dataset, where samples were taken from four existing monitoring wells (circa 1985) and nine Geoprobe® locations, and surface water/sediments were collected from an additional five locations (ENTECH, Inc., 1998B, 2003). A remedial investigation study in 2002 included a soil gas survey that was used to determine the extent of contamination, to identify TCE and other VOC hot spots, and to guide subsequent borings and well installation (BMT Entech, Inc., 2008C; ENTECH, Inc., 2003). Hydraulic conductivity data for the aquifer were estimated from slug tests conducted between 2004 and 2012 (BMT Designers and Planners, 2011B; BMT Entech, Inc., 2008B; ENTECH, Inc., 2003). In 2005, nine upstream and downstream monitoring wells were installed to measure VOC concentrations annually. In addition, groundwater potentiometric mapping and groundwater flow analyses were conducted (BMT Designers & Planners, 2011; BMT Entech, Inc., 2008C).

Although a more consistent groundwater sampling schedule was adopted in 2005, the lengthy time between collections and the limited analyte scope precluded in-depth analyses of changes in geochemistry, hydraulics, or VOC concentrations. Furthermore, sand screens at the base of the monitoring wells which prevent well-screen clogging and wear and tear on sampling pumps may have caused underestimation or exclusion of certain sediment-bound analytes (e.g. iron, ion species, minerals) in the groundwater samples (Menheer and Brigham, 1997; Vail, 2013). This is problematic because metal concentration data are of particular interest as certain types of iron in the soil and groundwater can promote the reduction of TCE (Davison and Seed, 1983; Elsner et al., 2004; He et al., 2008; Lee and Batchelor, 2002a, 2002b, 2003; Liang et al., 2009). All these data and assessments were used by the collaborators throughout the adaptive management process.

Remedial selection and biowall technology

The feasibility study step in the CERCLA process identified and considered several remediation action plan alternatives which are summarized in Table 3-2. The alternatives proposed by the consultant were based on the feasibility study and included several different approaches, each with increasing costs, intensity, and disruption to the area. Each of the remedial alternatives included a cost and health risk assessment (BMT Designers & Planners, 2011; BMT Entech, Inc., 2008B). Nine criteria were used in the selection process (BMT Entech Inc., 2009): overall protection of human health and the environment; compliance with Applicable or Relevant and Appropriate Requirements (ARARs) (meets laws and regulations set by CERCLA); long-term effectiveness and permanence of the action; reduction of toxicity, mobility, or volume of contaminants through treatment; short-term effectiveness; ability of the plan to be implemented; cost; acceptance by the state; and acceptance by the community. The biowall (Plan 4) was selected because mitigation was needed to decrease potential risk from the contaminants, and it was more cost-effective and easier to maintain than extraction (Plan 5). This decision was summarized in the Record of Decision (BMT Designers & Planners, 2011).

Table 3-2: Summary of the Beaverdam Road Landfill remediation action plan options (USDA-ARS, 2009; BMT Entech, Inc., 2008; BMT Designer & Planners, 2011B)

Plan	Activity	Description
1	No action	
2	Land use controls and monitoring	Use signs and fencing to enforce no-trespassing, with annual groundwater monitoring to ensure contamination is not getting worse or moving beyond the property boundaries.
3	Monitored natural attenuation, land use controls, and monitoring	In addition to the actions of Plan 2 (above), groundwater samples are periodically taken and analyzed to determine the extent of natural attenuation at the site.
4	Groundwater treatment via a mulch biowall permeable reactive barrier and land use controls	Build a structure approximately 0.5 to 0.9 m wide, 8 to 11 m deep, and 300 m long and implement land use controls from Plan 2.
5	Extraction, on-site treatment, recharge, and land use controls	Install extraction wells and pumps to extract groundwater. Send groundwater through a treatment system and use to recharge the aquifer.

Biowall technology is not a new remediation method for groundwater contamination. Over the last two decades, reactive barriers have been used extensively by the US Air Force, industry, and other entities to remediate a range of groundwater contaminants (AFCEE, 2008; FRTR, 2003; NATO/CCMS, 2001; Phillips et al., 2010; Vogan et al., 1999). Biowalls are a low-maintenance, green technology and are often a cost-effective remediation option. While biowalls do not address the contaminant source, they are designed to manage the contaminants of concern as they are released from the source and migrate through the subsurface. Thus, the biowall provides a large, reactive-surface area so that as groundwater passes through the structure, the released contaminants are sorbed and/or degraded (Figure 3-3).

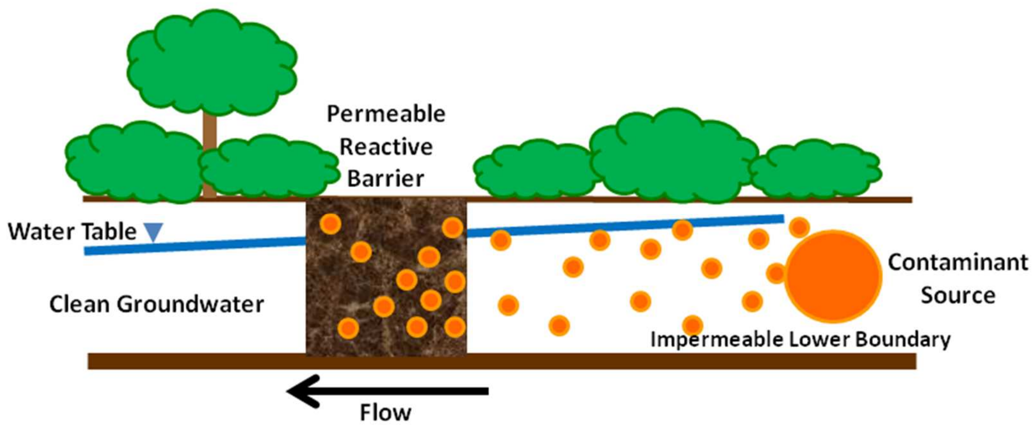

Figure 3-3: Biowall schematic

Longevity and robustness, however, are essential to the effectiveness of the biowall especially if the contaminant source lifetime and emission rate are unknown. Therefore, to ensure that a biowall remains low maintenance and economical, several factors need to be considered in implementation, including: the types of materials used and their degradation, the ratio of organic-to-inorganic material, degradation kinetics of the contaminant, water geochemistry, microbial community, hydraulic characteristics (i.e., porosity and flow), and structural dimensions which, in this case, were determined using transport modeling (Ahmad et al., 2007; Erto et al., 2011; FRTR, 2003; Groundwater Services, Inc., 2004; Phillips et al., 2010; Tratnyek et al., 1997; Vogan et al., 1998). Finally, another key aspect of this biowall was to attempt to utilize the native soil microbial population, specifically, those previously identified to degrade TCE.

Using adaptive management in biowall implementation and management

In addition to overseeing the installation of the engineered structure in July 2013, the consultant was also responsible for establishing a monitoring program to evaluate the biowall. Monitoring is required to assess the extent of transport, infiltration, and remediation of the site contaminants. However, the team wanted to establish a program that would be scientifically rigorous and would improve upon previous assessments of remediation activities, so an extensive literature review of similar remediation installations was conducted by the university partner (Michaelson 2012). The results of this literature review revealed that the final evaluation of some of the remediation efforts was based on insufficient or inappropriate sampling frequency based on flow rate, no established baseline, or poor sample collection protocol. Additionally, the university partner found issues with the final design of the biowall in some cases, such as insufficient residence time and leakage around the structure (FRTR, 2003; Gilbert et al., 2013; Groundwater Services, Inc., 2004; Lu et al. 2008; Vogan et al., 1999; Wilkin et al., 2005). While it is not possible to understand fully the limitations or other constraints placed on the studies from literature, in hindsight, many of these issues could have been resolved with continued scrutiny of the monitoring program employed to judge performance. Those remediation efforts may still have been successful, but the protocol implemented to gauge success was faulty and not necessarily designed to measure success.

The in-depth historical assessment and concurrent laboratory experiments married passive and active adaptive management styles and allowed the partners to avoid previous shortcomings during the implementation of the ROD. The outcomes of the experiments and assessments were discussed during the quarterly meetings and were incorporated into the biowall design and/or monitoring plan. CERCLA requires an efficacy assessment review every five years and a minimum 30-year commitment of groundwater monitoring to assure public safety. However, the partners determined that it was not appropriate to wait for the first five-year review to suggest changes to the monitoring plan or to initiate planning for future structural modifications based on the outcome of the scientific experiments (i.e., bioaugmentation, biostimulation, ZVI injection). Thus, refinements took place during the first 5-year period, in anticipation of the first review.

The decision points to either maintain the status quo or initiate a change in either the monitoring plan or biowall structure occurred during the institutional meetings and were vetted in subsequent emails and reports. Several intertwining points were deliberated prior to a decision: 1) the stability of the analytical parameter under consideration for change based on literature and historical site data; 2) using this information, how the change would affect the contaminant concentrations relative to the MCLs; and 3) the feasibility of the desired change from economic, structural, and practical perspectives. Specific changes are discussed below.

Changes to the biowall design

To determine the biowall fill material appropriate for achieving the site remediation goals, the academic partner conducted experiments investigating the type and mixture ratios of mulch and compost based on an extensive literature review (Niño de Guzmán et al., 2018a). ZVI and glycerol were also considered for their potential to improve TCE degradation; ZVI has been extensively studied in this capacity while the use of glycerol as a carbon source in this role has not been widely studied (Atashgahi et al., 2017; Gillham and O'Hannesin, 1994; Kouznetsova et al., 2007; Wilkin et al., 2003). These experiments were run in parallel so that the responses could be compared directly. Results showed that extra carbon and ZVI enhanced TCE disappearance, however, the quantity of iron filings needed to affect a change was impractically large. The findings were compiled and discussed with the partners. Based on these discussions, the consultant chose a mixture of 40% mulch generated partially from the trees and brush cleared to install the wall and 30% compost generated at the BARC compost research facility which provided the extra carbon and was readily accessible. The remainder of the wall consisted of concrete-grade sand and small gravel which facilitated flow and provided structural stability.

Transect wells were not a part of the original biowall design, but were added after assessing historical data. Following numerous discussions, the partners agreed that the locations of some downstream monitoring wells were not ideally situated to capture groundwater exiting the biowall. Thus, new transect wells were installed to monitor the migration of the plume hotspot as it moves through the biowall and the filtered groundwater as it exits the downstream side. Of the six wells, four were located upstream of the biowall, one of which was installed on the periphery, while the two remaining wells were located on the other outside edge and 0.36 m downstream. The inclusion of the additional monitoring groundwater wells has been invaluable for determining background analyte concentrations and will be a crucial component for high-quality, long-term data analysis in subsequent years as evidenced elsewhere (FRTR, 2003; Gilbert et al., 2013; Groundwater Services, Inc., 2004; Lu et al., 2008).

Updates to the field testing and monitoring plan

It was important to employ site-tailored questions and analyses to ensure that site remediation is successful. This baseline assessment was a critical step and provided the needed information for the biowall design and the restructuring of the monitoring plan. From this assessment, criteria were established to evaluate the success of the biowall, including contaminant movement, retention, and/or degradation and to consider the evolution of the site. The sampling number, schedule, and locations were also examined and adjusted to accommodate the needs of the modeling exercises. As a result, the sampling regime of the monitoring wells was changed from annually to quarterly beginning in 2014.

Since the installation of the biowall in 2013, the consultant has compiled quarterly reports of the data from the sampling activities. The initial reports, prior to biowall installation, were an exhaustive list of physical, geochemical, and chemical parameters measured in each sample to establish an extensive baseline. After a year, the partners observed that some of the parameters were quite stable and could be measured less frequently, while others provided superfluous information and could be eliminated from the list of analyses, reducing analytical costs. For example, nitrate and nitrite were removed from the analyte list one year after the biowall was installed because they were not detected in any of the biowall well samples. Sulfide analysis was also discontinued based on the likelihood that iron (II) sulfide formation was occurring inside the biowall, a precipitate unable to be captured in the collected water samples. On the other hand, methane, ethane, and ethene were added to the analyte list of the transect wells after the fourth quarter sampling event in order to improve monitoring of full degradation and methanogenesis. This type of data and analysis review provided savings financially and in human capital and allowed the partners to focus on efforts which could provide more fruitful analyses.

Adaptive management and microbial populations

Since installation of the biowall, TCE levels have decreased approximately 90% in the groundwater that passed through the structure, although levels of certain degradation products have increased. For instance, from March 2014 to September 2016, the vinyl chloride concentrations downstream of the biowall increased from 13 µg/L to approximately 20 µg/L while the ethylene concentrations at the same locations increased from not detected to approximately 5 µg/L (Niño de Guzmán et al., 2018a). The presence of ethylene is auspicious as it is the fully dechlorinated, non-toxic final degradation product, while the buildup of vinyl chloride is of concern due to its known toxicity. To combat the growing concentration of vinyl chloride strategically, field sampling and laboratory analyses have continued; the data are being used to inform the next generation of experiments to investigate and promote vinyl chloride degradation, among other objectives. Nonetheless, the hazard quotient of the site has decreased 88% (Niño de Guzmán et al., 2018a).

In addition to determining the composition of the biowall, the batch reactor experiments explored potential remedies to future degradation issues (addition of ZVI and/or glycerol) and insight into potential interaction concerns. The addition of ZVI shavings instigated sufficient reducing conditions to decrease but not completely prohibit vinyl chloride formation. However, ZVI shavings in concert with glycerol actually increased vinyl chloride production (Niño de Guzmán et al., 2018a). As previously mentioned, the impetus for including glycerol in these experiments was to explore its use as an easily accessible source of carbohydrates for the microbial population, to encourage their proliferation inside the biowall material and the biotic degradation of TCE. In the course of field sampling, the group discovered that the biowall material was aging faster than anticipated, possibly due to a combination of microbial activity and natural decay. Unfortunately, due to its effect on the production of vinyl chloride when coupled with iron, glycerol was found not to be the best material to use as a "sacrificial" carbohydrate-rich substance to slow the biological degradation of the biowall. This suggests that a different carbohydrate-containing material should be investigated.

Groundwater and soil samples were collected before and after biowall installation as well as from the biowall structure after one year later to monitor and to catalog the microbial community. Next generation DNA sequencing was employed to index the different bacteria present in high, moderate, and no contamination zones; nested PCR assays were used to specifically search for members of the *Dehalococcoides* genus, able to completely dechlorinate TCE. PCR assays were also used to search for the presence of three different functional genes previously found necessary for the dehalogenation of chlorinated ethenes (Fung et al., 2007; Holmes et al., 2006). The goal is to consider individuals or communities discovered in the samples for bioaugmentation of the biowall to increase the degradation capability and longevity of the structure. Additional feasibility experiments are underway to examine this issue as well as the effects of ZVI exposure on the indigenous microbial community.

Conclusions

As part of the Superfund program, the Beaver Dam Road Landfill remediation project required USDA, UMD, and the Consultant to work in concert for the successful implementation of the US EPA action plan. The trilateral approach taken by the team facilitated capture of the broadest remedial scope, while the use of adaptive management ensured that the action and monitoring plans remained organized and flexible. Communication between entities, though less formal, allowed for more free-flowing, frank dialogue. It is important to note that aside from their designated roles, the USDA research organization and the Consultant provided invaluable professional and real-world insight necessary to design experiments and to advise the UMD graduate students. This type of communication experience is often not available in a university environment either through the classroom or research projects.

The detailed historic data assessment in conjunction with the laboratory experiments to inform the final biowall design and monitoring plan has enabled this project to overcome or avoid several common pitfalls uncovered during the extensive literature review. From a regulatory perspective, this long-term project thus far has been a success. The biowall is working as intended to reduce the groundwater TCE concentration to the MCL downstream of the structure. Data assessment and research continue to evolve to address the dynamic system and are consistently used to inform future decisions concerning biowall adjustments and the monitoring regime. The major project changes in the beginning, i.e., monitoring well placement and chemical analyses, were the result of the impartial evaluation of historic and present data. The extension of the project from the original scope to include microbial community analysis is expected to generate results that will provide insight into structural biostimulation activities and the maturation of the site and structure.

The scope of this project is unique. As new information becomes available and technological advancements in the engineering and scientific fields are made, we anticipate that further refinements to the monitoring plan will be implemented. The use of a hybrid adaptive management method as a way to facilitate a constructive collaboration process is recommended in other similarly large projects to take advantage of the distinctive synergy that can be created though each participant area of expertise.

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References

[ATSDR] Agency for Toxic Substances and Disease Registry, 2006. Toxicological profile for Vinyl Chloride. U.S. Department of Health and Human Services, Public Health Service, Georgia. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=282&tid=51 (accessed 09 May 2018).

ATSDR, 2014. Toxicological profile for Trichloroethylene (TCE) (Draft for Public Comment). U.S. Department of Health and Human Services, Public Health Service, Georgia. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=173&tid=30 (accessed 09 May 2018).

Ahmad, F., McGuire, T.M., Lee, R.S., Becvar, E., 2007. Considerations for the design of organic mulch permeable reactive barriers. Remed. J. 18, 59-72. https://doi.org/10.1002/rem.20151

Airgas, 2004. Material safety data sheet: Ethylene. Airgas, Pennsylvania. Document number: 001022. https://www.airgas.com/msds/001022.pdf (accessed 09 May 2018).

BMT Designers & Planners, 2011a. US EPA Superfund record of decision: Beaver Dam Road landfill (BARC 27). Contract number 53-3K06-3-1900. *https://semspub.epa.gov/work/03/2162006.pdf* (accessed 09 May 2018).

BMT Designers & Planners, 2011b. Draft letter report for supplemental investigations at the BDRLF. Arlington (VA), USA: U.S. Department of Agriculture-Agricultural Research Service. Contract number 53-3K06-3-1900.

BMT Entech, Inc., 2008a. BARC Fact Sheet: Beaver Dam Road Landfill (BARC 27). Document accession number P-03-0137.

BMT Entech, Inc., 2008b. Feasibility study report for the Beaverdam Road landfill. Contract number 53-3K06-3-1900.

BMT Entech, Inc., 2008c. Remedial investigation for Beaverdam Road landfill. Contract number 53-3K06-3-1900.

BMT Entech, Inc., 2009. CERCLA proposed plan for the Beaver Dam Road landfill site. Document accession number D-19-0010.

BMT, 2014. Beaverdam road landfill: Biowall performance monitoring plan. Updated December 2014.

Davison, W., Seed, G., 1983. The kinetics of the oxidation of ferrous iron in synthetic and natural waters. Geochim. Cosmochim. Ac. 47, 67-69. https://doi.org/10.1016/0016-7037(83)90091-1

Elsner, M., Schwartzenbach, R.P., Haderlein, S.B., 2004. Reactivity of Fe(II)-bearing minerals toward reductive transformation of organic contaminants. Environ. Sci. Technol. 38, 799-807. https://doi.org/ 10.1021/es0345569

ENTECH, Inc., 1998a. Baseline Groundwater Sampling, After-Action Report: BARC 6 (Biodegradable Site), BARC 12 (Chemical Disposal Pits), and BARC 27 (Beaver Dam Road Landfill). Contract number 53-3K15-5-9083.

ENTECH, Inc., 1998b. Site screening work plan: BARC 27, BARC 41, EPIC 31, ENTECH 15, ENTECH M12, ENTECH R1, ENTECH R13. Contract number 53-3K15-5-9083.

ENTECH, Inc., 2003. Remedial investigation/feasibility study work plan for the Beaver Dam Road Landfill. Contract number 53-3K15-0-1200.

Erto, A., Lancia, A., Bortone, I., Di Nardo, A., Di Natale, M., Musmarra, D., 2011. A procedure to design a permeable adsorptive barrier (PAB) for contaminated groundwater remediation. J. Environ. Manage. 92, 23-30. https://doi.org/10.1016/j.jenvman.2010.07.044

Fung, J.M., Morris, R.M., Adrian, L., Zinder, S.H., 2007. Expression of reductive dehalogenase genes in *Dehalococcoides ethenogenes* strain 195 growing on tetrachloroethene, trichloroethene, or 2,3-dichlorophenol. Appl. Environ. Microb. 73, 4439-4445. https://doi.org/ 10.1128/AEM.00215-07

[FRTR] Federal Remediation Technologies Roundtable, 2003. Abstracts of Remediation Case Studies Volume 7. U.S. EPA/National Service Center for

Environmental Publications, Ohio. https://www.epa.gov/sites/production/files/2015-04/documents/abstractsvol7.pdf (accessed 09 May 2018).
Gilbert, O., Cortina, J.L., de Pablo, J., Ayora, C., 2013. Performance of a field-scale permeable reactive barrier based on organic substrate and zero-valent iron for in situ remediation of acid mine drainage. Environ. Sci. Pollut. Res. 20, 7854-7862. https://doi.org/10.1007/s11356-013-1507-2
Gillham, R.W., O'Hannesin, S.F., 1994. Enhanced degradation of halogenated aliphatics by zero-valent iron. Ground Water 32, 958-967. https://doi.org/10.1111/j.1745-6584.1994.tb00935.x
Gregory, R., Ohlson, D., Arvai, J., 2006. Deconstructing adaptive management: Criteria for applications to environmental management. Ecol. Appl. 16, 2411-2425.
Groundwater Services, Inc., 2004. Report for full-scale mulch wall treatment of chlorinated hydrocarbon-impacted groundwater. Groundwater Services, Inc., Texas. Contract number F41624-97-C-8020. DTIC: ADA422621. https://clu-in.org/download/contaminantfocus/dnapl/Treatment_Technologies/Mulch_Biowall.pdf (accessed 09 May 2018).
He, Y.T., Wilson, J.T., Wilkin, R.T., 2008. Transformation of reactive iron minerals in a permeable reactive barrier (biowall) used to treat TCE in groundwater. Environ. Sci. Technol. 42, 6690-6696. https://doi.org/10.1021/es8010354
Holmes, V.F., He, J., Lee, P.K.H., Alvarez-Cohen, L., 2006. Discrimination of multiple *Dehalococcoides* strains in a trichloroethene enrichment by quantification of their reductive dehalogenase genes. Appl. Environ. Microb. 72, 5877-5883. https://doi.org/10.1128/AEM.00516-06
Kouznetsova, I., Bayer, P., Ebert, M., Finkel, M., 2007. Modelling the long-term performance of zero-valent iron using a spatio-temporal approach for iron aging. J. Contam. Hydrol. 90, 58-80. https://doi.org/10.1016/j.jconhyd.2006.09.014
Lee, W., Batchelor, B., 2002a. Abiotic reductive dechlorination of chlorinated ethylenes by iron-bearing soil minerals. 1. Pyrite and magnetite. Environ. Sci. Technol. 36, 5147-5154. https://doi.org/10.1021/es025836b
Lee ,W., Batchelor, B., 2002b. Abiotic reductive dechlorination of chlorinated ethylenes by iron-bearing soil minerals. 2. Green rust. Environ. Sci. Technol. 36, 5348-5354. https://doi.org/10.1021/es0258374
Lee, W., Batchelor, B., 2003. Reductive capacity of natural reductants. Environ. Sci. Technol. 37, 535-541. https://doi.org/10.1021/es025830m
Liang, X., Philp, R.P., Butler, EC., 2009. Kinetic and isotope analyses of tetrachloroethylene and trichloroethylene degradation by model Fe(II)-bearing minerals. Chemosphere 75, 63-69. https://doi.org/10.1016/j.chemosphere.2008.11.042
Lu, X., Wilson, J.T., Shen, H., Henry, B.M., Kampbell, D.H., 2008. Remediation of TCE-contaminated groundwater by a permeable reactive barrier filled with plant mulch (biowall). J. Environ. Sci. Heal. A 43, 24-35. https://doi.org/ 10.1080/10934520701750421

Menheer, M.A., Brigham, M.E., 1997. Ground-water sampling methods and quality-control data for the Red River of the North Basin, Minnesota, North Dakota, and South Dakota, 1993-95. U.S. Geological Survey Water-Resources Investigations Report 96-4317. Contribution from the National Water-Quality Assessment Program. U.S. Geological Survey Branch of Information Services, Colorado.

Michaelson, D.E., 2012. Reevaluating the CERCLA process: A case study at Beaverdam landfill (Master's thesis). Retrieved from the Digital Repository at the University of Maryland. URI: http://hdl.handle.net/1903/13552

Niño de Guzmán, G.T., Hapeman, C.J., Millner, P.D., McConnell, L.L., Jackson, D., Kindig, D., Torrents, A., 2018a. Using a high-organic matter biowall to treat a trichloroethylene plume at the Beaver Dam Road landfill. Environ. Sci. Pollut. Res. 25, 8735-8746. https://doi.org/10.1007/s11356-017-1137-1

Niño de Guzmán, G.T., Hapeman, C.J., Millner, P.D., Kjellerup, B.V., Torrents, A., 2018b. Presence of organohalide-respiring bacteria in and around a permeable reactive barrier at a trichloroethylene-contaminated Superfund site. *In review*.

[NATO/CCMS] North Atlantic Treaty Organization/Committee on the Challenges of Modern Society, 2001. Evaluation of demonstrated and emerging technologies for the treatment of contaminated land and groundwater (Phase III), 2000 Annual Report. EPA 542-R-01-001.

Pacini, V.A., Ingallinella, A.M., Sanguinetti, G., 2005. Removal of iron and manganese using biological roughing up flow filtration technology. Water Res. 39, 4463-4475. https://doi.org/ 10.1016/j.watres.2005.08.027

Parsons Infrastructure & Technology Group, Inc., 2008. Technical protocol for enhanced anaerobic bioremediation using permeable mulch biowalls and bioreactors. Air Force Center for Engineering and the Environment Technical Directorate May 2008. https://clu-in.org/download/techfocus/prb/Final-Biowall-Protocol-05-08.pdf (accessed 09 May 2018).

Phillips, D.L., Van Nooten, T., Bastiaens, L., Russell, M.I., Dickson, K., Plant, S., Ahad, J.M.E., Newton, T., Elliot, T., Kalinm, R.M., 2010. Ten year performance evaluation of a field-scale zero-valent iron permeable reactive barrier installed to remediate trichloroethene contaminated groundwater. Environ. Sci. Technol. 44, 3861-3869. https://doi.org/10.1021/es902737t

Tratnyek, P.G., Johnson, T.L., Scherer, M.M., Eykholt, G.R., 1997. Remediating groundwater with zero-valent metals: Chemical considerations in barrier design. Ground Water Monit. R. 17, 108-114. https://doi.org/10.1111/j.1745-6592.1997.tb01270.x

[USDA-ARS] U.S. Department of Agriculture-Agricultural Research Service, 2012. Beltsville Agricultural Research Center Screening Investigations of Potential Areas of Concern. https://www.ars.usda.gov/sp2UserFiles/Place/80000000/CERCLA/CERCLA-2012/AreasofConcern.pdf (accessed 09 May 2018).

[US EPA] U.S. Environmental Protection Agency, 1995. National primary drinking water regulations: Cis- and trans-1,2-dichloroethylene. US EPA, Washington, D.C. EPA 811-F-95-004.

US EPA, 2009a. National primary drinking water regulations. http://water.epa.gov/drink/contaminants/upload/mcl-2.pdf (accessed 09 May 2018).
US EPA, 2009b. Superfund Information Systems: Cleanup Activities at Beltsville Agricultural Research Center (USDA). https://cumulis.epa.gov/supercpad/SiteProfiles/index.cfm?fuseaction=second.schedule&id=0300415 (accessed 09 May 2018).
US EPA, 2011. Superfund: CERCLA Overview. http://www.epa.gov/superfund/policy/cercla.htm (accessed 09 May 2018).
US EPA, 2013. What is Superfund? https://www.epa.gov/superfund/what-superfund (accessed 09 May 2018).
Vail, J., 2013. SESD operating procedure: Groundwater sampling. SESDPROC-301-R3. Region 4, US Environmental Protection Agency, Science and Ecosystem Support Division, Georgia. https://www.epa.gov/sites/production/files/2015-06/documents/Groundwater-Sampling.pdf (accessed 09 May 2018).
Vogan, J.L., Focht, R.M., Clark, D.K., Graham, S.L., 1999. Performance evaluation of a permeable reactive barrier for remediation of dissolved chlorinated solvents in groundwater. J. Hazard. Mater. 68, 97-108. https://doi.org/10.1016/S0304-3894(99)00033-3
Wilkin, R.T., Puls, R.W., Sewell, G.W., 2003. Long-term performance of permeable reactive barriers using zero-valent iron: Geochemical and microbiological effects. Ground Water 41, 493-503.

Chapter 5: Conclusions and future work

Conclusions

With the discovery of TCE at a concentration >400 µg/L, the Beaver Dam Road landfill was placed in the Superfund program to ensure the remediation of the TCE-contaminated groundwater and to prevent/circumvent further damage to human and environmental health. Per the Program's regulations, the US EPA oversaw the remediation plan and the USDA-ARS was charged with creating the remediation and monitoring plans. The University of Maryland, College Park and BMT Designers and Planners (Consultant) collaborated to initiate a series of experiments to determine the composition of the biowall's fill-material. The research and findings presented in Chapters 2 through 4 are summarized below.

Construction and monitoring of the biowall

Ultimately, a 305 m long by 0.7 to 0.8 m wide by 5.5 to 7 m deep biowall with an unamended 4:3 mulch/compost fill-material mixture was implemented in the field. This mixture was able to remove approximately 90% of the TCE concentration from the groundwater passing through the structure and decrease the overall hazard quotient by 88%. Though the concentration of ethene was observed to be increasing, so was the concentration of VC. While the presence of both is indicative of reductive dechlorination, VC is very toxic and means that complete dechlorination is not being achieved. The degradation of VC into ethylene or some other nontoxic byproduct is a high priority.

Investigating the use of ZVI and glycerol as possible biowall fill-material amendments provided valuable information and direction for future experiments. Adding ZVI to the reactors improved the reducing conditions and the measured VC concentrations were five to ten times lower than those reactors containing some measure of glycerol, though slightly above the MCL. Because a biologically active biowall is desired, the antimicrobial properties of ZVI were a concern. To understand if the native microbial population would react negatively to ZVI, a flow-through column study was designed. Fortunately, ZVI did not appear to negatively impact the dechlorinating population in these studies. Given the steady increase of VC in the field (specifically within and downstream of the biowall), the introduction of ZVI or other material may become necessary to force better reducing conditions. At this time, the current microbial community able to contribute to VC degradation is not present in a high enough concentration to biologically combat this issue. By improving the living conditions within the biowall (using biostimulation) for select microbial communities, a corresponding increase in dechlorination rates should be evident.

Though the inclusion of glycerol was intended to provide the native microbial community with an easily accessible carbohydrate-containing molecule, this material produced noticeably higher VC concentrations when used in concert with ZVI in batch experiments. A future iteration of the batch reactor experiment would do well to observe this material, or a similar one, on its own with the biowall fill-material. The rapid deterioration of the biowall's bioavailable organic carbon fraction indicates high microbial activity and calls for the introduction of a sacrificial carbon source in order to keep up with the carbon demand.

Biowall environment versus native microbial population

Continuously-fed, flow-through column reactors were constructed to understand what, if any, would be the impact of a highly-reducing material (ZVI) on a specialized, dechlorinating microbial community (SDC-9). It was assumed that the added (antimicrobial) stress would necessitate biostimulation in order to prevent total community collapse and/or death. qPCR of the 16S rRNA extracted from the columns did not reveal a statistically significant difference between columns with or without ZVI, nor with or without biostimulation (modified-RAMM media vs. groundwater-only) though the addition of ZVI (without biostimulation) did decrease the overall population; the functional gene *bvcA* (responsible for VC degradation to ethene) remained dominant in all reactors, followed by *vcrA* (TCE to ethene), and lastly *tceA* (TCE to VC). While there was no significant difference with regards to overall *Dehalococcoides* population between the reactors containing the biowall material fed with modified-RAMM media (Reactor 2), and the reactor containing ZVI and fed with groundwater (reactor 3), the quantity of the functional genes *tceA*, *vcrA*, and *bvcA* were always significantly different. At the tested ZVI dose (5 mL/L), biostimulation would not be required to maintain a *Dehalococcoides* population. These experimental results will be utilized in future remediation and/or biowall expansion plans to fully employ the natural resources at the study site.

In conjunction with the flow-through columns, qPCR and next-generation sequencing heavily used to survey the site's indigenous microbial community for microbes previously identified to be positively associated (directly or indirectly) with the degradation of TCE or any of its degradation products. Methanogens, acetogens, sulfate-reducers, and chlorinated aliphatic hydrocarbon-metabolizers were positively identified making way for the possible cultivation of this community for use in biowall bioaugmentation efforts. Though *Dehalococcoides* was also found at the site, it was in quantities below the 0.1% cutoff quantity. This may mean that this particular population is ripe for targeting with some specific growth media. The field measurements indicate that the current geochemical conditions (e.g. Fe^{2+}concentration, pH, sulfate concentration) and the slowly increasing ethene concentration inside the biowall can support (and are supporting) this important dechlorinating microbe albeit at low concentrations.

Institution of a hybrid adaptive management system to organize site remediation actions and maintain flexibility for future research

As part of the Superfund program, the Beaver Dam Road Landfill remediation project required collaboration between the USDA, UMD, and the Consultant to achieve successful implementation of the US EPA action plan. To capture the broadest remedial scope and allow for the incorporation of continuously developing experiments, adaptive management was used to ensure that the action and monitoring plans remained organized and flexible. Less formal, though professional, communication between entities fostered free-flowing, frank dialogue.

This collaborative environment has also helped this project to overcome or avoid several common pitfalls uncovered during the extensive literature review. The open, trusting relationship facilitated the detailed, impartial historic data assessment to take place in conjunction with the laboratory experiments to inform the final biowall design and monitoring plan (i.e. monitoring well placement, addition/elimination of chemical analyses). From a regulatory perspective, this long-term project thus far has been a success as the biowall is working as intended. Data assessment and research continue to evolve to address the dynamic biowall system and developing remediation needs (such as VC reduction).

The scope of this project is unique. Especially important has been the consideration of time; the careful planning of experiments and the resulting period in which they are run must work in conjunction with the needs of the site and how quickly a solution is required. As new information becomes available and technological advancements in the engineering and scientific fields are made, we anticipate that further refinements to the monitoring plan will be implemented. The use of a hybrid adaptive management method as a way to facilitate a constructive collaboration process is recommended.

Future work

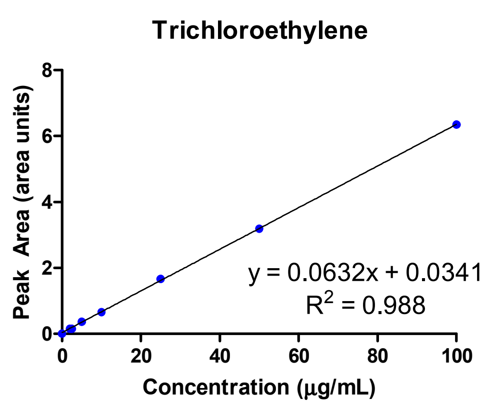
Previous chapters of the body of work identify numerous issues related to TCE abiotic and biotic degradation that require attention and should be explored with future research. These topics include:

1. Completely reduce the vinyl chloride concentration downstream of the structure to below its MCL.
2. Incorporate a sacrificial carbon-containing molecule to a) slow the biological degradation of the organic fill-material, and b) provide an easily accessible carbon source for population proliferation.
3. Cultivate select native soil microbial communities for use in future bioaugmentation efforts to bolster the abiotic TCE degradation processes.
4. Establish the dechlorination rates of the native microbial community to better direct bioaugmentation/biostimulation efforts.
5. Explore other biostimulation media/methods to increase the native *Dehalococcoides* population as well as related "helpful" microbial communities for full TCE degradation.
6. Decontaminate the spent biowall fill-material for non-hazardous disposal.

Appendices

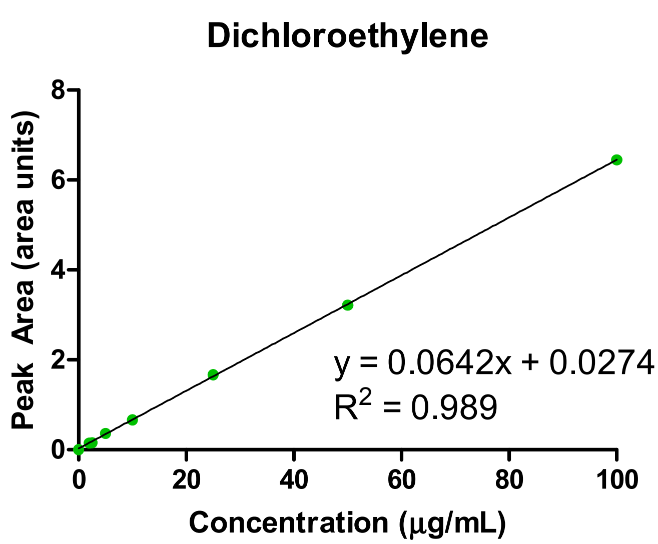
Appendix A1: Trichloroethylene standard curve raw data and graph

X (ppm)	0	2.0	2.5	5.0	10.0	25.0	50.0	100.0
	0.00	0.14	0.13	0.80	0.93	1.90	3.70	7.20
			0.13	0.21	0.90	2.00	2.70	7.50
		0.12		0.63	1.20	2.00	3.10	7.30
		0.21	0.14	0.35	0.62	1.50	3.10	6.20
		0.16	0.13	0.30	0.63	1.50	4.00	6.40
		0.19	0.19	0.34	0.73	1.60	2.70	6.40
		0.17	0.15	0.33	0.66	1.60	3.20	5.70
		0.15	0.11	0.29	0.65	1.70	3.60	5.50
		0.12	0.13	0.33	0.61	1.60	3.70	5.60
		0.10		0.52	0.67	1.60	3.30	5.70
		0.14	0.19	0.44	0.49	1.40	3.30	5.70
		0.11	0.14	0.64	0.65	1.80	3.20	6.20
		0.17	0.17	0.34	0.70	1.70	3.10	6.50
		0.14	0.17	0.28	0.65	1.70	1.60	6.60
			0.15	0.33	0.63	1.60	2.70	6.60
			0.13	0.34	0.64	1.60	3.40	6.90
			0.17	0.30	0.71	1.40	3.40	6.60
			0.12	0.29	0.60	1.80	3.00	5.90
			0.20	0.52	0.53	1.80	3.30	5.50
			0.12	0.37	0.72	1.70	3.30	5.60
			0.17	0.67	0.54	1.50	2.90	6.40
			0.12	0.31	0.62	1.70	3.00	7.00
			0.15	0.30	0.67	1.30	3.30	6.90
			0.19	0.34	0.68	1.70		6.40
			0.17	0.33	0.65	1.60		6.60
			0.15	0.29	0.67	1.60		6.20
				0.25	0.71	1.70		6.60
				0.55	0.61	1.70		5.70
				0.38	0.57			5.90
				0.36	0.66			6.70
				0.53	0.62			6.70
				0.41	0.62			7.10
				0.34	0.67			6.80
				0.36	0.63			6.50
				0.32	0.68			6.70
				0.33	0.49			6.00
				0.31	0.70			5.30
				0.31	0.64			
				0.30	0.68			
				0.31				
				0.29				
				0.33				
				0.33				
				0.33				



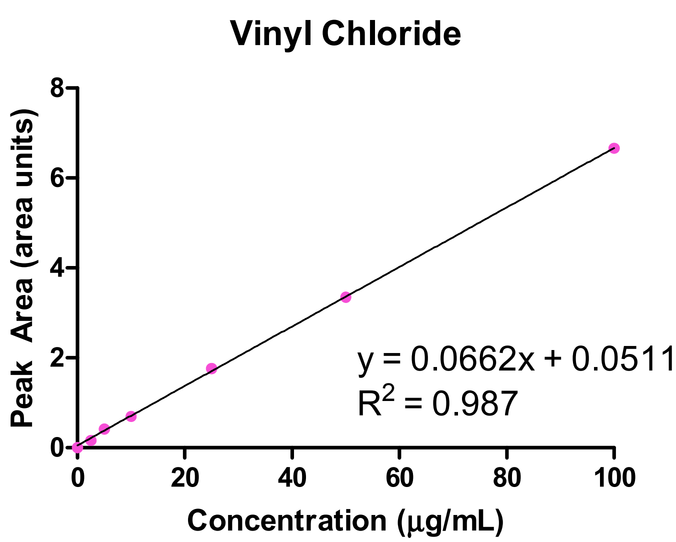
Appendix A2: *cis*-Dichloroethylene standard curve raw data and graph

X (ppm)	0	2.0	2.5	5.0	10.0	25.0	50.0	100.0
	0.00	0.14	0.15	0.73	0.92	1.90	3.70	7.40
			0.13	0.49	0.66	1.60	4.00	6.50
		0.12		0.49	0.66	1.60	3.70	5.60
		0.22	0.13	0.53	0.69	1.60		5.80
		0.16	0.15	0.51	0.51	1.40	1.70	6.30
		0.19	0.19	0.22	0.66	1.80	2.80	6.60
		0.17	0.15	0.46	0.71	1.70	2.80	6.70
		0.14	0.11	0.38	0.65	1.70	3.50	6.60
		0.13	0.14	0.39	0.64	1.60	3.20	7.00
		0.11		0.41	0.65	1.60	2.80	6.70
		0.14	0.19	0.37	0.70	2.00	3.00	6.00
		0.11	0.15	0.60	0.62	1.50	2.90	5.60
		0.16	0.18	0.62	0.53	1.70	3.10	7.70
		0.14	0.15	0.65	0.89		3.30	6.70
			0.15	0.35	0.66	1.40	3.30	5.60
			0.14	0.35	0.66	1.80		5.80
			0.18	0.35	0.72	1.80	3.40	6.50
				0.35	0.55	1.70	3.30	7.00
			0.20	0.33	0.63	1.50	3.00	7.00
			0.12	0.32	0.68	1.70	3.10	6.50
			0.16	0.30	0.67	2.00	3.60	6.70
			0.14	0.33	0.65	1.60	3.10	6.20
			0.13	0.33	0.67	1.60		6.60
			0.18	0.30	0.71		3.40	5.80
			0.12	0.31	0.61	1.30	3.30	7.50
			0.16	0.34	0.56	1.70	3.30	6.70
			0.15	0.30	1.30	1.60		5.80
				0.33	0.75	1.60		6.00
				0.29	0.62	1.70		6.70
				0.30	0.66	1.70		6.70
				0.33	0.63			7.20
				0.35	0.62			6.80
				0.30	0.67			6.60
				0.27	0.64			6.70
				0.31	0.68			6.00
				0.31	0.49			5.40
				0.33	0.69			5.90
				0.34	0.64			
				0.34	0.68			
				0.28				
				0.26				
				0.32				
				0.30				
				0.34				



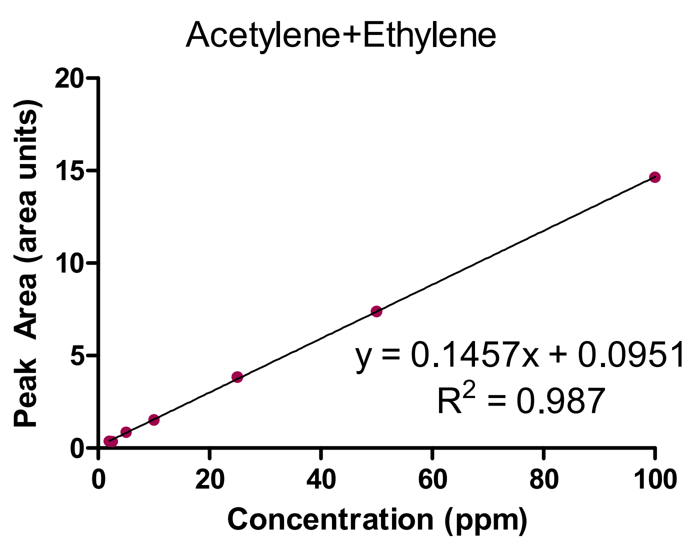
Appendix A3: Vinyl chloride standard curve raw data and graph

X (ppm)	0	2.0	2.5	5.0	10.0	25.0	50.0	100.0
	0.00	0.15	0.15	0.74	0.93	2.00	3.80	7.50
		0.14	0.16	0.51	0.78	2.10	4.10	7.90
		0.22	0.18	0.50	12.60	2.00	3.80	7.80
		0.18	0.13	0.55	0.72	1.80	3.20	6.70
		0.19	0.13	0.52	0.53	14.70	3.00	7.00
		0.17	0.15	2341.30	0.68	1.70	3.70	7.10
		0.14	0.14	0.82	0.72	1.50	3.40	6.20
		0.15	0.20	938.60	0.67	1.80	17.30	20.30
		0.12	0.13	0.37	0.67	1.80	3.10	5.80
		0.16	0.12	0.35	0.66	1.70	1.70	6.30
		0.12	0.20	0.36	0.72	1.70	2.80	6.80
		0.18	0.16	0.32	0.64	1.70	3.40	6.80
		0.15	0.17	0.30	0.56	1.70	3.40	6.80
			0.18	0.36	0.93	16.30	16.20	7.00
			0.16	0.32	0.81		3.60	6.70
			0.14	0.35	13.50	1.40		6.10
			0.20	5.60	0.76	1.90	3.10	5.70
			0.13	0.73	0.58	1.90	3.30	13.70
			0.17	944.60	0.65	1.80	3.30	5.80
			0.15	0.32	0.71	1.50	16.80	6.50
			0.15	0.36	0.69	1.70	3.20	7.10
			0.19	0.35	1.60	1.80		7.10
			0.18	0.34	0.70	13.90	3.00	6.60
			0.15	0.27	0.72		3.00	6.70
				0.33	0.65	1.40	3.40	6.20
				0.33	0.60	1.70		6.70
				0.35	1.30	1.70		5.90
				1.30	0.90	1.60		14.80
				0.70	12.30	1.70		6.00
				846.00	0.71	1.70		6.80
				0.35	0.66			6.80
				0.34	0.65			7.20
				0.36	0.70			6.90
				0.31	0.66			6.70
				0.27	1.20			6.70
				0.34	0.50			6.10
				0.31	0.72			5.50
				0.36	0.66			
				0.60	0.70			
				0.75				
				0.59				
				14.20				
				12.30				
				12.00				



Appendix A4: Ethene + Acetylene standard curve raw data and graph

X (ppm)	0	2.0	2.5	5.0	10.0	25.0	50.0	100.0
		0.35	0.36	1.54	2.10	4.40	8.60	16.90
			0.35	1.10	2.00	4.80	9.30	17.60
		0.33	0.42	1.10	1.80	4.60	8.50	17.60
		0.50	0.30	1.20	1.60	3.80	6.80	15.20
		0.40	0.32	1.10	1.60	3.70	6.60	12.70
		0.47	0.34	0.78	1.60	3.70	8.20	13.10
		0.42	0.32	1.10	1.20	3.30	7.60	14.00
		0.35	0.45	0.87	1.50	4.00	7.20	15.30
		0.34	0.29	0.88	1.60	4.00	7.00	15.10
		0.28	0.28	0.96	1.50	3.90	3.90	14.70
		0.34	0.46	0.86	1.50	3.70	6.20	15.70
		0.28	0.36	1.35	1.50	3.70	7.70	14.80
		0.41	0.37	0.90	1.60	3.70	7.60	13.50
		0.34	0.37	0.74	1.40	3.90	7.60	12.80
			0.34	0.78	1.30		8.00	15.80
			0.31	0.79	1.60	3.30		12.90
			0.46	0.71	1.60	4.20	7.00	13.10
			0.29	0.66	1.70	4.20	7.40	14.50
			0.38	0.81	1.30	3.90	7.40	15.80
			0.32	0.75	1.50	3.40	7.70	15.70
			0.28	0.80	1.60	3.80	7.10	14.40
			0.41	0.74	1.50	3.90		15.00
			0.39	0.78	1.50	3.80	6.70	13.80
			0.36	0.80	1.60		6.70	14.80
				0.68	1.60	3.10	7.60	13.20
				0.57	1.40	3.80		16.00
				0.78	1.30	3.70		14.10
				0.72	1.80	3.70		13.30
				0.81	1.50	3.80		13.60
				1.47	1.50	3.80		15.00
				1.52	1.50			15.10
				0.88	1.50			16.10
				0.83	1.60			15.10
				0.88	1.50			14.80
				0.81	1.60			14.80
				0.80	1.10			13.60
				0.71	1.60			12.30
				0.81	1.50			
				0.79	1.60			
				0.71				
				0.61				
				0.76				
				0.75				
				0.80				



Appendix A5: Trichloroethylene concentration (µg/mL) measured in the headspace of batch reactors.

Group 1	Days	M4C3 G0 Fe0	M4C3 G0 Fe5	M4C3 G0 Fe 10	M4C3 G10 Fe10	M4C3 G30 Fe10	M1C1 G0 Fe0	M1C1 G10 Fe10	M1C1 G0 Fe10	Sand Spike	Soil Spike
~~June 9*~~	~~1*~~										
16-Jun	4	0.219 0.184	0.317 0.281		0.317 0.255 0.264 0.281	0.388 0.379 0.379	0.281 0.299	0.219 0.175 0.192	0.335 0.379	0.539 0.450	0.308 0.326
23-Jun	11		~~0.086~~ 0.166 0.112		0.228 0.192 0.210	0.166 0.166 0.166	0.210 0.201	0.139 0.121 0.148			0.237
30-Jun	18	0.201 0.192 0.192	0.139 0.139 0.139	0.095 0.086 0.082	0.157 0.175 0.166	0.157 0.148 0.139	0.201 0.192 0.201	0.130 0.130 0.130	0.237 0.246 0.237	0.450 0.432 0.459	0.255 0.255 0.246
7-Jul	25	~~0.095~~ 0.166 0.112		~~0.040~~		0.130 0.130 0.130	0.201 0.201	0.095 0.104		0.423 0.468	0.272 0.281
14-Jul	32	0.104 0.095 0.112			0.065 0.104 ~~0.021~~	0.112 0.130 0.130	0.184 0.192 0.192		0.175 0.184 0.184		0.104 0.246 0.130
21-Jul	39	0.112 0.104 0.104	0.064 0.075 0.064	0.043 0.044 0.042	0.073 0.080 0.086	0.104 0.104 0.095	0.157 0.148 0.148	0.074 0.072 0.075	0.112 0.130 0.130	0.308 0.308 0.317	0.184 0.210 0.210
29-Jul	47		0.037 0.038 0.040				0.139 0.148 0.139		0.130 0.121 0.130	0.361 0.317 0.290	0.139 0.139
5-Aug	53	0.067 0.065 0.078	0.024 0.025 0.026	0.032 0.032 0.032	0.059 0.062 0.056	0.080 0.068 0.074	0.130 0.121 0.112	0.046 0.052 0.049	0.121 0.104 0.112	0.272 0.281 0.228	0.148 0.157 0.148
11-Aug	59	0.057 0.060 0.044 ~~0.036~~	0.021 0.020	0.026 0.025 0.024	0.048 0.052 0.053		0.121 0.121 0.121	0.048 0.046 0.035	0.095 0.095 0.086	0.255 0.246 0.237	0.139 0.148 0.139
19-Aug	67		0.021	0.026 ~~0.020~~ 0.026	0.048 0.050 0.045		0.104 0.112 0.121	0.039 0.044 0.034	0.095 0.095 0.095	0.264 0.272 0.317	0.175 0.157 0.184
26-Aug	74			0.024 0.024 0.022	0.046 ~~0.040~~ 0.047	0.056 0.056 0.052	0.104 0.112 0.112	0.032 0.033 0.033	0.086 0.083 0.080	0.308 0.272 0.272	0.157 0.148 0.139
2-Sep	80			0.023 0.029 0.025	0.040 ~~0.047~~ 0.040	0.049 0.050 0.040	0.095 ~~0.121~~ 0.095	0.032 0.032 0.032	0.069 0.074 0.074	0.281 0.272 0.264	0.130 0.148 0.175
8-Sep	86			0.024 0.023 0.025	0.040 0.044 0.040	0.040 0.047 0.040	0.104 0.104 ~~0.139~~	0.033 0.029 0.026	0.068 0.062 0.078	0.281 0.272 0.255	0.139 0.139 0.157
16-Sep	94			0.028 0.022 0.026	0.042 0.040 0.041	0.021 0.024	0.112 0.130 0.112	0.032 0.028 0.027	0.066 0.068 0.067	0.308 0.264 0.290	
24-Sep	102			0.029 0.027	~~0.024~~ 0.039 0.039		0.112 0.121 0.112		0.028 0.024 0.041	0.264 0.281 0.281	
3-Oct	111				0.024 0.027 0.025		0.083 ~~0.073~~ 0.085		0.031 0.031 0.027	0.219 0.210 0.210	0.112 0.112
16-Oct	124			0.020	0.025 0.023 0.022		0.095 0.081 0.085		0.022	0.255 0.246 0.201	0.112 0.121 0.121
21-Oct	129				0.023 0.025 0.025		0.080 0.076 0.072			0.219 0.219 0.201	0.112 0.112 0.130
30-Oct	138				0.023 0.025		0.086 0.074 0.095			0.272 0.264 0.299	0.157 0.157 0.166
6-Nov	144				0.021 0.021 0.023		0.074 0.085 0.083			0.237 0.246 0.246	0.112 0.112 0.157
13-Nov	151				0.022		0.076 0.079 0.086			0.210 0.237 0.237	
20-Nov	158				0.021		0.073 0.085 ~~0.112~~			0.272 0.264	0.104 0.121 0.112
5-Feb	593									0.299 0.290 0.317	0.175 0.175 0.166

Group 2	Days	M4C3 G0 Fe0	M4C3 G0 Fe5	M4C3 G0 Fe 10	M4C3 G10 Fe10	M4C3 G30 Fe10	M1C1 G0 Fe0	M1C1 G10 Fe10	M1C1 G0 Fe10	Sand Spike	Soil Spike
~~June 11*~~	~~1*~~										
18-Jun	4	~~0.563~~ ~~0.545~~ ~~0.563~~	0.210 0.201 0.201	0.166 0.201 0.237	0.237 0.210 0.246	0.308 0.308 0.264	0.326 0.272 0.264	0.166 0.104 0.121	0.246 0.210	0.352 0.335	0.192 0.219 0.219
25-Jun	11	0.139	0.166 0.166 0.175		0.219 0.175 0.184	0.219 0.228 0.228	~~0.166~~ ~~0.121~~	0.166 0.157 0.157	0.192 0.192 0.201	0.450 0.432 0.441	0.379 0.343 0.308
2-Jul	18	0.130 0.148 0.139	0.112 0.121 0.112	0.157 0.139 0.148	0.130 0.121 0.130	0.166 0.157	0.201 0.210 0.184	0.139 0.157 0.139	0.175 0.192 0.184	0.432 0.450 0.441	0.299 0.317 0.317
9-Jul	25	0.121 0.112	0.104 0.095	0.121		0.148 0.139 0.130	0.184 0.201 0.192	0.148 0.148 0.148	0.157 0.175 0.166	0.415 0.379 0.423	0.308 0.326
16-Jul	32	0.064 0.065 0.066	0.068 0.071 0.071			0.104 0.112 0.112	0.192 0.175 0.166	0.121 0.121 0.112	0.139 0.139 0.139	0.415 0.423 0.388	
24-Jul	40		0.056 0.055 0.054	0.075 0.065 0.070	0.049 0.051 0.050	0.072 0.070 0.069	~~0.095~~ ~~0.112~~ ~~0.104~~	0.067 0.077 0.072	0.112 0.112	0.335 0.326 0.352	0.237 0.201 0.219
31-Jul	47				0.052 0.054 0.055		0.130 0.148 0.130			0.201 0.246	
7-Aug	53			0.055 0.046 0.052	0.037 0.035 0.032	0.037 0.058 0.043	0.112 0.104 0.104	0.056 0.074 0.071	0.080 0.080 0.081	0.272 0.255 0.237	0.184 0.192 0.184
13-Aug	59			0.050 0.048	0.042 0.036 0.034	0.045 0.050 0.039	0.104 0.095 0.104	0.057 0.063 0.064	0.075 0.072 0.080	0.281 0.272 0.246	0.219 0.192 0.177
20-Aug	66			0.041 0.037 0.040	0.029 0.028 0.025		0.095 0.095 0.082	0.047 0.051 0.050	0.077 0.057 0.069	0.255 0.264 0.264	0.201 0.246 0.237
27-Aug	73			0.032 0.036 0.037	0.022 0.021	0.024 0.022 0.024	~~0.073~~ ~~0.086~~ 0.104	0.047 0.051 0.047	0.049 0.065 0.062	0.246 0.246	0.201 0.184 0.192
4-Sep	80			0.038 0.037 0.040	0.024 0.024 0.024	0.022 0.027 0.024	0.112 0.112 0.104		0.060 0.055 0.056		
10-Sep	86			0.025 0.031 0.029	0.021	0.020	0.095 0.086 0.095	0.046 0.052 0.041	0.046 0.057 0.049	0.264 0.228 0.237	0.184 0.184 0.192
17-Sep	93			0.024 0.023			0.095 0.086	0.032 0.027 0.037	0.044 0.038 0.044	0.246 0.219 0.237	0.219 0.192 0.192
25-Sep	101			0.024 0.026 0.024			0.095 ~~0.055~~ 0.095	0.036 0.040	0.041 0.039 0.043	0.228 0.246	0.192 0.228 0.210
6-Oct	112						0.063 0.072 0.064	0.024 0.024	0.039 0.027 0.025	0.228 0.201 0.175	0.175 0.166 0.148
17-Oct	123						0.074 0.073	0.024	0.028	0.246 0.246	0.166 0.192 0.184
23-Oct	129						0.081 0.077 0.071	0.024 0.020		0.255 0.175 0.219	0.192 0.201 0.237
31-Oct	137										
7-Nov	143						0.072 0.068 0.075			0.228 0.246 0.219	0.192 0.201 0.210
14-Nov	150						0.063 0.069 0.070			0.228 0.210	
20-Nov	156						0.081 0.073 0.084			0.237 0.246	
Feb-29	616									0.104 0.121 0.085	0.044 0.076 0.082

Group 3	Days	M4C3 G0 Fe0	M4C3 G0 Fe5	M4C3 G0 Fe 10	M4C3 G10 Fe10	M4C3 G30 Fe10	M1C1 G0 Fe0	M1C1 G10 Fe10	M1C1 G0 Fe10	Sand Spike	Soil Spike
~~June 12*~~	~~1*~~										
19-Jun	4		0.184 0.121	0.317 0.272	0.184 0.192 0.192	0.361 0.397 0.415	0.299 0.272 0.264	0.157 0.139 0.175	0.237 0.272 0.228	0.574 0.477 0.477	0.352 0.397
26-Jun	11			0.192 0.184	0.139 0.112 ~~0.192~~	0.255 0.255 0.246	0.228 0.237	0.112 0.112 0.112	0.219 0.192 0.192		0.228 0.352
3-Jul	18	0.166 0.166 0.157	0.148 0.148 0.157	0.139 0.148 0.148	0.157	0.184 0.210 0.192	0.175 0.184 0.184	0.112 0.112 0.112	0.157 0.166 0.166	0.468 0.423 0.459	0.317 0.326 0.317
10-Jul	25	0.148 0.157 0.157	0.130 0.130 0.130	0.148 0.139		0.175 0.175 0.184	0.175 0.184 0.175	0.121 0.104 0.121	0.166 0.148 0.157	0.415 0.423 0.450	0.290 0.308 0.272
17-Jul	32	0.121 0.121 0.112	0.104 0.095 0.104	0.112 0.121 0.121	0.095 0.104 0.095	0.148 0.148 0.157	0.157 0.148 0.148	0.095 0.121	0.148 0.139 0.130	0.388 0.388 0.388	0.255 0.281
25-Jul	40	0.086 0.095 0.085	0.058 ~~0.082~~ 0.048 0.045	0.078 0.082 0.079		0.071 0.057 0.086	0.112 0.121	0.066	0.121 0.130 0.121	0.326 0.246 0.290	0.264 0.246 0.237
2-Aug	47	~~0.058~~ 0.071 0.072	0.057 0.053 0.058	0.072 0.068 0.066	0.060 0.058 0.051	0.056 0.064 0.056	0.104 0.095 0.095	0.064 0.068		0.272 0.281	0.192 0.201 0.201
8-Aug	53	0.022 0.020 0.021	0.041 0.040 0.033	0.064 0.066 0.064	0.061 0.053 0.056	0.095 0.095 0.077	0.104 0.104 0.084	0.062 0.063	0.095 0.095 0.104	0.326 0.246 0.281	0.228 0.210 0.219
15-Aug	60			0.059 0.051 0.058	0.045 0.044 0.047	0.063 0.056 0.064	0.095 0.095 0.104		0.086 0.078 0.086	0.246 0.228 0.299	0.210 0.210
21-Aug	66			0.050 0.049 0.041					0.080 0.082 0.081	0.308 0.281 0.326	
29-Aug	74			0.040 0.040 0.041	0.030 0.031 0.037	0.051 0.054 0.054	0.079 0.095 0.104	0.055 0.048 0.047	0.095 0.077 0.080	0.255 0.290 0.272	0.201 0.157 0.201
5-Sep	80			~~0.030~~ 0.039 0.035	0.027 0.028 0.024	0.040 0.038 0.029	0.086 0.086	0.040 0.041 0.043	0.075 0.065 0.072		0.192 0.201 0.219
12-Sep	87			0.036 0.036 0.037	0.023 0.022 0.021	0.030 0.040 0.033	0.086 0.086 0.083	0.043 0.045 0.039	0.073 0.072 0.072	0.272 0.299 0.299	0.210 0.219 0.219
18-Sep	93			0.029 0.025 0.032		0.039 0.030 0.032	0.081 0.086 0.073	0.041 0.039 0.035	0.077 0.068		0.228 0.210 0.192
26-Sep	101			0.030 0.028 0.023			0.086 0.080 0.076	0.030 0.030 0.030	0.053 0.064 0.055	0.255 0.237	0.175 0.184 0.166
10-Oct	115						0.065 ~~0.057~~ 0.074	0.024 0.026 0.025	0.048 0.044 0.041	0.219 0.210 0.184	0.148 0.157 0.148
17-Oct	122						0.061 0.063 0.067	0.028 0.025 0.029	0.043 0.044 0.037	0.246 0.246	0.166 0.192 0.184
7-Nov	142						0.060 0.067 0.066	0.022 0.021 0.024	0.028 0.027 0.027	0.272 0.281 0.255	0.210 0.184 0.184
14-Nov	149						0.064 0.058 0.062		0.020	0.255 0.228 0.246	0.184 0.192 0.184

Appendix A6: *cis*- Dichloroethylene concentration (μg/mL) measured in the headspace of batch reactors.

Group 1	Days	M4C3 G0 Fe0			M4C3 G0 Fe5			M4C3 G0 Fe10	M4C3 G10 Fe10	M4C3 G30 Fe10	M1C1 G0 Fe0	M1C1 G10 Fe10	M1C1 G0 Fe10	Sand Spike	Soil Spike
~~9-Jun~~	~~1*~~														
16-Jun	4														
23-Jun	11														
30-Jun	18				~~0.282~~	~~0.282~~	~~0.289~~								
7-Jul	25														
14-Jul	32														
21-Jul	39														
29-Jul	47														
5-Aug	53														
11-Aug	59														
19-Aug	67	0.032	0.030	0.022											
26-Aug	74	0.027	0.023	0.025											
2-Sep	80	0.027	0.018	0.020	0.023	0.021	0.025								
8-Sep	86	0.018	0.021	0.020	0.021		0.020								
16-Sep	94	0.020	0.025	0.026	0.027	0.025	0.026								
24-Sep	102	0.021	0.025	0.020	0.024	0.026	0.024								
3-Oct	111	0.015	0.019		0.015	0.020	0.020								
16-Oct	124	0.016	0.018	0.016	0.020	0.018									
21-Oct	129					0.015									
30-Oct	138	0.016	0.014	0.017	0.015	0.015	0.019								
6-Nov	144		0.021	0.018	0.021	0.017	0.018								
13-Nov	151	0.016	0.016	0.015	0.016	0.015	0.017								
20-Nov	158	0.017	0.015		0.016	0.016	0.015								

Group 2	Days	M4C3 G0 Fe0			M4C3 G0 Fe5			M4C3 G0 Fe10	M4C3 G10 Fe10	M4C3 G30 Fe10	M1C1 G0 Fe0	M1C1 G10 Fe10			M1C1 G0 Fe10	Sand Spike	Soil Spike
~~11-Jun~~	~~1*~~											~~0.033~~	~~0.029~~	~~0.036~~			
18-Jun	5																
25-Jun	12				~~0.334~~	~~0.334~~	~~0.366~~										
2-Jul	19																
9-Jul	26																
16-Jul	33	0.014		0.015													
24-Jul	41	0.038	0.038	0.038													
31-Jul	48	0.032	0.044	0.043													
7-Aug	54	0.033	0.034	0.031	0.023	0.023	0.020										
13-Aug	60	0.034	0.034	0.031	0.016	0.016	0.014										
20-Aug	67	0.028	0.029	0.023	0.016	0.016	0.018										
27-Aug	74	0.027	0.025	0.023													
4-Sep	81	0.030	0.032	0.028	0.014	0.016											
10-Sep	87	0.023	0.028	0.021													
17-Sep	94	0.020	0.016	0.022													
25-Sep	102	0.023	0.025	0.021													
6-Oct	113		0.014	0.016													
17-Oct	124	0.018	0.014														
23-Oct	130																
31-Oct	138			0.015													
7-Nov	144	0.016	0.015	0.018													
14-Nov	151	0.014	0.019	0.016													
20-Nov	157	0.021	0.014	0.017													

Group 3	Days	M4C3 G0 Fe0			M4C3 G0 Fe5				M4C3 G0 Fe10	M4C3 G10 Fe10	M4C3 G30 Fe10	M1C1 G0 Fe0	M1C1 G10 Fe10	M1C1 G0 Fe10	Sand Spike	Soil Spike
~~12-Jun~~	~~1*~~															
19-Jun	5															
26-Jun	12															
3-Jul	19															
10-Jul	26															
17-Jul	33															
25-Jul	41															
2-Aug	49															
8-Aug	55	0.027	0.025	0.027												
15-Aug	62	0.027	0.027	0.027	0.034	0.031	0.029									
21-Aug	68	0.023	0.031	0.026	0.023	0.025	0.032									
29-Aug	76	0.025	0.028	0.028	0.027	0.031	0.028									
5-Sep	83	0.026	0.026	0.025	0.031	~~0.049~~	0.027	0.029								
12-Sep	90	0.023	0.027	0.023	0.025	0.027	0.029									
18-Sep	96	0.021	0.019	0.021	0.023	0.025	0.025									
26-Sep	104	0.023	0.021	0.022	0.021	0.024	0.024									
10-Oct	118	0.016							~~0.014~~							
17-Oct	125	0.02	0.02	0.021	0.019	0.016	0.016									
7-Nov	146	0.018	0.018	0.017	0.016	0.016	0.018									
14-Nov	153	0.016	0.016	0.018	0.015		0.014									

Appendix A7: Vinyl chloride concentration (μg/mL) measured in the headspace of batch reactors.

Group1	Days	M4C3 G0 Fe0				M4C3 G0 Fe5			M4C3 G0 Fe 10			M4C3 G10 Fe10				M4C3 G30 Fe10				M1C1 G0 Fe0			M1C1 G10 Fe10				M1C1 G0 Fe10					Sand Spike				Soil Spike			
~~9-Jun~~	~~1*~~	~~0.498~~	~~0.656~~	~~0.321~~	~~0.196~~															~~0.175~~	~~0.405~~	~~0.793~~								~~0.007~~	0.014								
~~16-Jun~~	~~4*~~					~~4.327~~	~~0.071~~	~~0.143~~	~~0.664~~	~~0.389~~	~~0.349~~	~~0.179~~	~~0.385~~	~~0.756~~	~~0.539~~	~~0.083~~	~~0.284~~	~~0.381~~					~~0.361~~	~~0.309~~	~~0.341~~		~~0.147~~	~~0.196~~	~~0.135~~			~~0.260~~	~~0.054~~	~~0.107~~	~~0.192~~	~~0.861~~	~~0.377~~	~~0.401~~	
23-Jun	11						0.007	0.005				~~0.011~~	0.009	0.008		0.031	0.031	0.031					0.018	~~0.013~~	0.017											~~0.018~~	0.006		0.008
30-Jun	18											0.009	0.011	0.010		0.031	0.031	0.028					0.021	0.024	0.024											0.014	0.015	0.015	
7-Jul	25															0.028	0.027	0.029					~~0.014~~	0.022	0.024											~~0.008~~	0.015	0.017	
14-Jul	32											~~0.006~~		0.012		0.025	0.029	0.031																		0.007	~~0.013~~	0.008	
21-Jul	39											0.010	0.012	0.012		0.038	0.035	~~0.029~~					~~0.018~~	0.013	0.013														
29-Jul	47																~~0.006~~															0.005				0.007		0.008	
5-Aug	53											0.013	0.014	0.013		0.038	0.034	0.037					0.012	0.014	0.013								0.005			0.008	0.008	0.008	
11-Aug	59											0.012	0.014	0.015									0.014	0.013	0.010														
19-Aug	67											0.017	0.020	0.018									0.014	~~0.017~~	0.013									0.006					
26-Aug	74																						0.013	0.014	0.014							0.006							
2-Sep	80											~~0.015~~	0.021	0.021		0.046	0.046	0.042					0.015	0.014	0.014														
8-Sep	86											0.018	0.021	0.020		0.050	~~0.058~~	0.050					0.016	0.014	0.013														
16-Sep	94											~~0.021~~	0.025	0.026		~~0.062~~	~~0.035~~	~~0.038~~	~~0.034~~				0.016	0.016	~~0.014~~							0.006				0.006			
24-Sep	102											~~0.013~~	0.026	0.028			~~0.008~~		~~0.007~~					~~0.008~~	0.018	0.012								0.005					
3-Oct	111											0.017	0.018	0.019		0.050	0.046	0.050					0.012	0.010	0.014			0.005											
16-Oct	124					0.006						0.029	0.028	0.027		0.071	0.067	0.067					0.015	~~0.021~~	0.015		0.010	0.011	0.009										
21-Oct	129								0.007			0.021	0.026	0.023		0.058	0.058	~~0.075~~					0.016	0.017	0.015		0.010	0.010	0.008										
30-Oct	138							0.006	0.008			0.030	~~0.019~~	0.029		0.071	~~0.075~~	0.071					~~0.027~~	0.018	0.017		~~0.014~~	0.018	0.016					0.006					
6-Nov	144					0.007	0.006	0.007		~~0.006~~	0.011	~~0.027~~	0.031	0.032		0.075	0.075	0.079					0.022	0.025	~~0.032~~		~~0.017~~	0.015	0.015										
13-Nov	151					0.007	0.006	0.006	0.010	0.008	~~0.031~~	~~0.027~~	0.032	~~0.006~~		0.075	0.079	0.075					0.016	~~0.037~~	0.016		0.018	0.016	0.018										
20-Nov	158					0.008	0.008	0.008		0.007		0.034	0.032	0.037		0.087	~~0.075~~	0.091					0.030	~~0.022~~	0.030		0.012		~~0.007~~					0.005					

Group 2	Days	M4C3 G0 Fe0			M4C3 G0 Fe5			M4C3 G0 Fe 10			M4C3 G10 Fe10			M4C3 G30 Fe10				M1C1 G0 Fe0				M1C1 G10 Fe10			M1C1 G0 Fe10			Sand Spike			Soil Spike		
11-Jun	~~1*~~																																
18-Jun	~~5*~~	~~0.107~~	~~0.069~~	~~0.069~~	~~0.083~~	~~0.028~~	~~0.016~~	~~0.212~~	~~0.103~~	~~0.054~~	~~0.054~~	~~0.027~~	~~0.029~~	~~0.067~~	~~0.038~~	~~0.025~~		~~0.184~~	~~0.099~~	~~0.046~~	~~0.083~~	~~0.135~~	~~0.029~~	~~0.034~~	~~0.119~~	~~0.05~~	~~0.046~~	~~0.075~~	0.135	~~0.042~~	~~0.03~~	~~0.027~~	0.014
25-Jun	12										~~0.02~~	0.013	0.011	0.018	0.02	0.021						0.019	0.018	0.02				~~0.021~~	0.006	0.013	~~0.007~~		
2-Jul	19										0.017	0.017	0.016	~~0.038~~	0.023	0.019						0.018	0.021	0.018				0.011	~~0.007~~	0.011			
9-Jul	26													0.018	0.018	0.018						0.014	0.014	0.014				0.01	0.006				
16-Jul	33	~~0.01~~										0.008		0.036	0.037	0.038						0.017	0.016	0.015									
24-Jul	41										0.012	0.012	0.012	0.035	0.034	0.037						0.012	0.013	0.014									
31-Jul	48										0.016	0.017	0.016																				
7-Aug	54										0.016	0.015	0.012	0.033	~~0.054~~	0.038						0.015	0.019	0.018									
13-Aug	60							0.006			0.022	0.02	0.018	~~0.02~~	0.054	0.062	0.046					0.018	0.021	0.021									
20-Aug	67						0.005				0.019	0.02	0.016									0.018	0.019	0.02									
27-Aug	74								0.005		0.019	0.015	0.019	0.054	0.05	0.05						0.018	0.021	0.018									
4-Sep	81				0.006	~~0.012~~	0.006	0.006	0.005	0.006	0.025	0.029	0.025	0.058	0.075	0.067						0.029	0.021	0.032					~~0.008~~				
10-Sep	87				0.006	0.005					0.021	0.03	0.022	0.079	0.067	0.058						0.026	0.035	0.024									
17-Sep	94				0.006	~~0.009~~	0.006			0.006	0.021	0.03	0.027	0.071	0.075	0.075						~~0.019~~	~~0.018~~	~~0.029~~				0.018	0.013	0.012			
25-Sep	102				0.008	0.008	~~0.011~~	0.01	0.008	0.007	0.027	0.03	0.031	0.087	0.087	0.083				~~0.031~~		0.028	0.036		0.005								
6-Oct	113				~~0.007~~	0.011	0.012	0.006	0.008	0.006	0.03	0.035	0.035	0.087	0.087	0.091						0.032	0.031	0.029	0.008	0.006							
17-Oct	124				0.015	0.022	~~0.008~~	~~0.012~~	0.006	0.006	0.046	0.046	~~0.038~~	0.103	0.115	0.099						0.031	0.038	~~0.062~~	0.013	0.009	0.01						
23-Oct	130					~~0.005~~		0.006	0.007	0.007	~~0.016~~	0.05	0.038	0.103	0.087	0.127						0.038	0.038	~~0.058~~	0.011	0.013	0.011						
31-Oct	138				0.007	~~0.013~~	0.006	0.007	0.007	0.008	~~0.028~~	0.036	0.038	0.103																			
7-Nov	144				0.01	0.008	0.009	0.009	0.008	0.008	0.05	0.035	0.042	0.111	0.103	0.103						~~0.016~~	0.038	0.031	0.016	0.014	0.015						
14-Nov	151				~~0.021~~	0.01	0.011	~~0.018~~	0.01	0.013	0.05	0.071	0.046	0.115	0.115	0.115						~~0.027~~	0.05	0.042	0.014	0.014	0.017						
20-Nov	157				0.011	0.013	0.012	0.012	0.011	~~0.019~~	0.054	0.067	0.046	0.131	0.135	0.139						0.054	0.05	0.05	0.008	0.014	~~0.024~~						

Group 3	Days	M4C3 G0 Fe0			M4C3 G0 Fe5					M4C3 G0 Fe 10					M4C3 G10 Fe10				M4C3 G30 Fe10					M1C1 G0 Fe0			M1C1 G10 Fe10				M1C1 G0 Fe10			Sand Spike			Soil Spike		
~~12-Jun~~	~~1*~~	0.079	0.071	0.075																				~~0.123~~	~~0.024~~	~~0.023~~													
~~19-Jun~~	~~5*~~				~~0.042~~	~~0.115~~	~~0.563~~	~~0.135~~	~~0.123~~	~~0.139~~	~~0.119~~	~~0.099~~	~~0.031~~	~~0.034~~	~~0.155~~	~~0.046~~	~~0.091~~	~~0.05~~	~~0.075~~	~~0.03~~	~~0.019~~	~~0.033~~					~~0.103~~	~~0.05~~	~~0.058~~		~~0.021~~	~~0.017~~	~~0.007~~	~~0.091~~	~~0.022~~	~~0.025~~	~~0.067~~	~~0.042~~	~~0.046~~
26-Jun	12				0.006	0.006	~~0.009~~			0.011	0.018	0.017			0.018	0.016	0.02		0.019	0.02	0.021						0.013	0.012	0.014					0.028	0.029	0.027	~~0.01~~	0.015	0.017
3-Jul	19				0.008	0.008	~~0.006~~								~~0.079~~	0.011	0.013		0.029	0.031	~~0.024~~						0.018	0.02	0.02					~~0.03~~	0.017	0.016	0.015	0.014	0.014
10-Jul	26																		0.024	0.024	0.026						0.021	0.019	0.022										
17-Jul	33										0.008				~~0.037~~	~~0.046~~	~~0.127~~		0.032	0.031	0.031						0.022	0.027	~~0.01~~					0.021	0.021	0.022	0.011	0.013	
25-Jul	41														~~0.01~~				~~0.011~~	0.025	~~0.006~~	~~0.019~~	0.031						~~0.016~~	~~0.028~~				0.019	0.017	0.018	0.011	0.009	0.009
2-Aug	49											0.01			~~0.032~~	0.018	0.016		0.026	0.028	0.026						0.024	0.026	~~0.01~~								0.006		0.006
8-Aug	55										0.006	0.005			0.027	0.025	0.025		~~0.058~~	0.03	0.042							0.025	0.025		0.006	0.006	0.006	0.019	0.016	0.018	0.007	0.006	0.006
15-Aug	62									0.007	0.006	0.007			0.028	0.027	0.029		0.038		0.036	0.042						~~0.012~~			0.008	0.007	0.008	0.014	0.012	0.015			
21-Aug	68									0.007	0.011				~~0.027~~	~~0.019~~	~~0.016~~			~~0.034~~											0.009	0.009	0.008	0.016	0.014	0.016			
29-Aug	76									~~0.009~~	0.006	0.006			0.042	0.03	~~0.05~~		0.062	0.067	0.067						0.027	0.029	0.029		~~0.012~~	0.009	0.009	0.015	0.016	0.015			
5-Sep	83					0.009				~~0.005~~	0.008	0.01			0.042	0.046	~~0.031~~			0.062	0.058	~~0.05~~					0.025	0.027	0.028		0.01	0.009	0.01	~~0.006~~	~~0.006~~	~~0.008~~			
12-Sep	90									0.008	0.007	0.008			0.042	0.042	~~0.031~~		0.058	~~0.079~~	0.067						0.033	0.036	~~0.024~~		0.012	0.012	0.012	0.017	0.019	0.018			
18-Sep	96									~~0.012~~	~~0.006~~	0.008			0.038	0.038	~~0.062~~		~~0.095~~	0.071	0.075						~~0.038~~	0.029	0.031		0.015	0.013	~~0.029~~						
26-Sep	104					0.009				~~0.016~~	0.01	0.008			~~0.027~~	0.035	0.033		~~0.027~~	~~0.033~~							~~0.032~~	0.026	0.025		0.012	0.015	0.013		0.017	0.014			
10-Oct	118									0.007	0.008	0.01			0.042	0.034	~~0.024~~		0.062	0.067	0.058						0.035	0.034	0.035		0.016	0.015	0.014	0.013	0.012	0.011			
17-Oct	125				0.006	~~0.005~~	0.01			~~0.014~~	0.011	0.01			0.033	0.034	0.031		0.071	0.067	~~0.188~~						0.05	0.05	0.054		0.021	0.021	~~0.016~~						
7-Nov	146				0.008	0.008	0.009			~~0.019~~	0.01	0.012			0.038	0.038	~~0.071~~		~~0.071~~	0.079	0.083						0.083	~~0.095~~	0.079		0.027	0.026	0.027						
14-Nov	153				0.011	0.008	0.008			~~0.024~~	0.012	0.012			0.054	0.046	~~0.038~~		0.087	0.079	0.079						0.067	0.058	0.062		0.025	0.023	0.024						

Appendix A8: Ethene and acetylene concentraions (μg/mL) measured in the headspace of the batch reactors.

Group 1	Days	M4C3 G0 Fe0					M4C3 G0 Fe5			M4C3 G0 Fe 10				M4C3 G10 Fe10			
16-Jun	4	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00		0.01	0.01	0.01	0.01
23-Jun	11	0.00	0.00	0.00	0.00		0.00	0.01	0.01	0.00	0.00		0.00	0.03	0.03	0.03	
30-Jun	18	0.00	0.00	0.00			0.02	0.02	0.02	0.02	0.01	0.01		0.06	0.06	0.06	
7-Jul	25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	
14-Jul	32	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00		0.10	0.00	0.16	0.03
21-Jul	39	0.01	0.01	0.01			0.06	0.07	0.06	0.03	0.03	0.03		0.17	0.18	0.20	
29-Jul	47	0.00	0.00	0.00			0.15	0.16	0.17	0.00	0.02	0.01	0.03	0.00	0.00	0.00	
5-Aug	53	0.03	0.02	0.03			0.43	0.44	0.47	0.06	0.06	0.06		0.23	0.24	0.21	
11-Aug	59	0.05	0.05	0.04	0.03		1.01	1.01	1.00	0.10	0.10	0.10		0.22	0.25	0.25	
19-Aug	67	0.13	0.12	0.09	0.00		2.87	2.39	2.45	0.25	0.20	0.26		0.27	0.28	0.25	
26-Aug	74	0.30	0.26	0.28			5.05	4.87	4.89	0.56	0.55	0.50		0.28	0.25	0.28	
2-Sep	80	0.69	0.52	0.54			12.07	12.54	13.98	1.30	1.51	1.33		0.27	0.31	0.26	
8-Sep	86	0.87	0.99	0.97			21.88	13.90	20.03	2.54	2.24	2.66		0.28	0.30	0.28	
16-Sep	94	4.44	5.74	6.04			55.91	54.87	56.65	6.83	5.85	6.55		0.32	0.30	0.30	
24-Sep	102	8.78	12.16	10.26			72.57	78.08	74.66	17.76	12.78	14.49		0.21	0.33	0.34	
3-Oct	111	10.68	13.30				60.48	80.53	77.04	29.83	27.89	32.08		0.26	0.30	0.27	
16-Oct	124	23.38	28.56	28.17			101.87	95.16		66.17	65.10	59.28		0.33	0.32	0.31	
21-Oct	129	26.48	26.91	19.35			77.55	79.97	78.42	55.89	51.82	50.36		0.29	0.33	0.32	
30-Oct	138	47.90	40.79	46.34			99.94	101.84	123.30	69.93	72.61	70.20		0.36	0.32	0.38	
6-Nov	144	2.74	73.07	67.15			163.44	130.54	138.07	91.65	91.01	82.84		0.40	0.41	0.41	
13-Nov	151	63.14	65.13	61.33			134.41	126.34	139.85	90.53	74.56	0.43		0.33	0.42	0.42	
20-Nov	158	77.81	72.04	65.38			140.51	144.55	144.51	93.94	100.16	94.23		0.44	0.42	0.50	

Group 1	M4C3 G30 Fe10				M1C1 G0 Fe0			M1C1 G10 Fe10					M1C1 G0 Fe10			Sand Spike				Soil Spike			
16-Jun	0.03	0.03	0.03				0.00	0.01	0.01	0.01			0.00	0.00	0.00	0.00	0.00	0.00	0.00				
23-Jun	0.15	0.15	0.15					0.06	0.05	0.06			0.00	0.00	0.00	0.00	0.01	0.00	0.00			0.000	0.000
30-Jun	0.16	0.16	0.14		0.00	0.00	0.00	0.11	0.11	0.12			0.01	0.01	0.01								
7-Jul	0.16	0.16	0.17		0.00	0.00	0.00	0.09	0.13	0.14			0.00	0.01	0.00								
14-Jul	0.19	0.22	0.22		0.00	0.00	0.00	0.00	0.00	0.01			0.02	0.02	0.02								
21-Jul	0.25	0.24	0.23		0.00	0.00	0.00	0.15	0.14	0.15			0.02	0.02	0.02	0.00	0.00	0.00		0.00	0.00	0.004	
29-Jul	0.00	0.05	0.00		0.00	0.00	0.00	0.00	0.00	0.00			0.03	0.02	0.03	0.00							
5-Aug	0.36	0.32	0.35		0.00	0.00	0.00	0.16	0.17	0.17			0.03	0.03	0.03								
11-Aug	0.00		0.00		0.00	0.00	0.00	0.19	0.19	0.14			0.03	0.03	0.03	0.00	0.00	0.00					
19-Aug	0.00	0.00	0.00		0.00	0.00	0.00	0.20	0.23	0.18			0.04	0.04	0.04					0.00	0.00	0.001	
26-Aug	0.52	0.54	0.49		0.00	0.00	0.00	0.20	0.21	0.21			0.04	0.04	0.04					0.00	0.00	0.003	
2-Sep	0.53	0.55	0.46		0.00	0.00	0.00	0.25	0.24	0.24			0.04	0.04	0.04		0.00			0.00	0.00	0.005	
8-Sep	0.53	0.62	0.52		0.00	0.00	0.01	0.29	0.26	0.24			0.04	0.04	0.05	0.00	0.00	0.00		0.00	0.01	0.006	
16-Sep	0.63	0.34	0.39	0.33	0.00	0.01	0.00	0.38	0.36	0.34			0.05	0.05	0.05	0.00	0.00	0.00		0.00	0.00	0.001	
24-Sep		0.09	0.01	0.09	0.00	0.01	0.00	0.07		0.28	0.56	0.39	0.03	0.03	0.05	0.00	0.00	0.00		0.00	0.00	0.001	
3-Oct	0.47	0.44	0.47		0.01	0.01	0.01	0.75	0.70	0.93			0.06	0.06	0.06	0.00	0.00	0.00		0.00	0.01	0.005	
16-Oct	0.61	0.59	0.60		0.01	0.01	0.01	5.18	5.18	5.22			0.10	0.11	0.09	0.00	0.00	0.00		0.01	0.01	0.006	
21-Oct	0.51	0.49	0.62		0.01	0.00	0.01	9.73	10.08	9.56			0.10	0.10	0.08	0.00	0.00	0.00		0.00	0.00	0.006	
30-Oct	0.59	0.66	0.62		0.01	0.01	0.01	44.70	38.59	34.73			0.14	0.16	0.16	0.00	0.00	0.00		0.01	0.01	0.011	
6-Nov	0.64	0.64	0.66		0.01	0.01	0.01	89.81	99.29	90.65			0.17	0.16	0.16	0.00	0.00	0.00		0.01	0.01	0.012	
13-Nov	0.59	0.63	0.60		0.01	0.01	0.01	117.89	158.53	119.77			0.19	0.16	0.18	0.00	0.00	0.00		0.00	0.00	0.004	
20-Nov	0.69	0.59	0.73		0.01	0.01	0.01	300.78	220.06	206.26			0.13	0.02	0.08	0.00	0.00	0.00			0.01	0.014	0.013

Group 2	Days	M4C3 G0 Fe0					M4C3 G0 Fe5			M4C3 G0 Fe 10				M4C3 G10 Fe10		
18-Jun	5	1.94	2.00	2.12			0.01	0.00	0.00				0.01	0.13	0.12	0.13
25-Jun	12	0.09	0.66		3.74	0.48	0.02	0.02	0.02					0.28	0.23	0.24
2-Jul	19	1.22	1.38	1.32			0.04	0.04	0.04	0.03	0.03	0.03		0.35	0.34	0.36
9-Jul	26	0.21	0.50	1.39	1.34		0.09	0.07	0.06		0.04		0.01			
16-Jul	33	2.45	2.45	2.48			0.10	0.10	0.10						0.11	
24-Jul	41	3.28	3.27	3.38			0.21	0.20	0.20	0.07	0.06	0.06		0.57	0.59	0.60
31-Jul	48	3.99	5.46	5.41			0.03	0.03	0.03		0.03	0.02	0.06	0.85	0.89	0.92
7-Aug	54	6.30	6.47	5.97			1.45	1.54	1.35	0.12	0.10	0.12		0.96	0.90	0.85
13-Aug	60	9.76	9.81	9.10			2.23	2.41	2.04	0.28	0.15	0.21	0.21	1.64	1.47	1.39
20-Aug	67	13.21	13.86	11.36			6.35	5.88	7.07	0.40	0.36	0.39		1.96	1.98	1.72
27-Aug	74	19.18	20.44	17.91			11.25	12.85	12.01	0.73	0.82	0.84		2.68	2.13	2.58
4-Sep	81	35.44	39.37	35.37			28.37	35.09	31.37	2.26	2.14	2.35		4.97	5.05	4.87
10-Sep	87	37.64	46.38	34.31			39.83	38.34	31.16	3.16	3.68	3.56		5.51	6.04	5.70
17-Sep	94	39.51	38.19	51.90			46.30	49.97	52.31	8.70	6.85	7.62		7.67	8.56	7.80
25-Sep	102	78.69	82.53	75.74			67.93	72.65	59.58	18.61	21.73	19.84		13.44	12.65	11.26
6-Oct	113	57.10	72.14	73.37			65.99	64.89	70.94	25.95	29.96	26.99		17.88	20.89	22.27
17-Oct	124	113.22	97.43	84.45			90.97	92.46	109.17	44.84	43.06	51.98		34.98	34.90	32.00
23-Oct	130	93.30	49.85	52.95			7.43	83.95	74.22	41.81	44.91	52.07		13.87	36.07	33.81
31-Oct	138	71.64	93.54	109.05			103.79	94.68	83.22	58.92	50.93	58.29		34.35	36.90	38.77
7-Nov	144	144.11	132.00	152.59			137.29	107.67	117.53	75.18	65.06	67.51		60.05	53.83	50.33
14-Nov	151	139.25	172.41	148.40			142.69	135.20	123.91	86.28	78.41	91.66		68.71	103.69	68.34
20-Nov	157	206.52	148.36	168.01			150.30	145.81	148.36	89.26	87.23	95.99		122.28	99.42	95.12

Group 2	M4C3 G30 Fe10				M1C1 G0 Fe0			M1C1 G10 Fe10			M1C1 G0 Fe10			Sand Spike		Soil Spike		
18-Jun	0.04	0.04	0.04					0.04	0.03	0.04								
25-Jun	0.10	0.11	0.11					0.09	0.09	0.09	0.01	0.01	0.01					
2-Jul	0.22	0.18	0.17					0.10	0.11	0.10	0.01	0.01	0.01					
9-Jul	0.26	0.25	0.24					0.15	0.15	0.15	0.02	0.02	0.02					
16-Jul	0.34	0.35	0.35					0.19	0.19	0.18	0.02	0.02	0.02					
24-Jul	0.35	0.36	0.36					0.14	0.16	0.15								
31-Jul																		
7-Aug	0.36	0.58	0.45					0.20	0.26	0.25	0.02	0.02	0.02					
13-Aug	0.18	0.62	0.66	0.53				0.26	0.29	0.29	0.02	0.02	0.02					
20-Aug								0.30	0.32	0.32	0.03	0.02	0.03					
27-Aug	0.59	0.58	0.61					0.40	0.44	0.41	0.02	0.03	0.03					
4-Sep	0.74	0.90	0.81					0.89	0.81	0.98	0.03	0.03	0.03	0.04	0.04			
10-Sep	0.90	0.77	0.69					1.73	1.92	1.59	0.03	0.04	0.03					
17-Sep	0.75	0.81	0.78					3.26	2.82	3.92	0.04	0.03	0.04					
25-Sep	0.85	0.85	0.83				11.34	10.55	11.56	0.00	0.04	0.04	0.05					0.01
6-Oct	0.80	0.80	0.83		0.01	0.01	0.01	36.18	36.82	30.76	0.08	0.05	0.04			0.01	0.01	0.01
17-Oct	0.93	1.01	0.90		0.01	0.01	0.01	145.21	148.96	199.30	0.09	0.07	0.07			0.01	0.01	0.01
23-Oct	0.89	0.75	1.10		0.01	0.01	0.01	338.37	301.40	~~496.692*~~	0.10	0.11	0.10			0.01	0.01	0.01
31-Oct	0.84																	
7-Nov	0.92	0.84	0.85		0.01	0.01	0.01	226.98	434.34	388.87	0.16	0.15	0.16			0.01	0.01	0.01
14-Nov	0.96	0.94	0.94		0.01	0.01	0.01	211.27	683.05	472.75	0.15	0.15	0.16					
20-Nov	1.05	1.08	1.11		0.02	0.01	0.02	610.14	584.06	550.56	0.10	0.17	0.19		0.01			

Group 3	Days	M4C3 G0 Fe0				M4C3 G0 Fe5					M4C3 G0 Fe 10			M4C3 G10 Fe10			
19-Jun	6										0.02	0.85	0.72	1.46	1.35	1.54	1.57
26-Jun	13										0.60	2.40	2.29	2.31	1.84	3.14	
3-Jul	20	5.53	5.64	5.48		0.04	0.04	0.05			4.41	4.73	4.85	3.40	0.67	1.08	
10-Jul	27	7.21	7.42	7.37		0.07	0.07	0.07			2.49	6.24	6.12		0.39		
17-Jul	34	8.36	7.89	7.57		0.08	0.08	0.08			7.12	7.53	7.53	5.52	5.97	5.61	
25-Jul	42	0.54	8.07	8.35	7.80			0.10	0.06	0.05	8.22	8.36	8.03	1.19			
2-Aug	49	10.19	12.63	12.30		0.13	0.11	0.12			11.32	10.62	10.39	7.57	7.26	6.39	
8-Aug	55	14.25	13.39	13.90		0.18	0.17	0.15			14.62	14.20	14.04	10.29	9.07	9.57	
15-Aug	62	15.94	16.05	16.44		0.34	0.31	0.30			18.63	16.70	18.83	11.33	11.11	11.59	
21-Aug	68	18.45	24.67	20.84		0.44	0.47	0.60			25.10	24.64	20.50	12.02	8.25	7.46	
29-Aug	76	27.27	29.71	30.42		1.12	1.29	1.16			28.07	29.69	30.39	15.21	15.22	17.22	
5-Sep	82	36.55	37.48	36.21		2.67	4.18	2.32	2.46		33.61	43.69	40.87	18.85	18.80	17.50	
12-Sep	89	39.44	45.61	38.24		4.42	4.85	5.16			56.63	57.64	58.07	24.82	23.57	23.17	
18-Sep	95	40.15	37.63	39.90		7.93	8.55	8.61			57.64	52.32	60.73	30.20	23.83	30.89	
26-Sep	103	55.88	51.55	54.28		19.30	21.79	21.72			76.63	73.99	62.44	40.92	50.23	48.51	
10-Oct	117	53.68	52.87	43.75		33.76	41.58	41.92			59.47	74.26	61.39	70.58	75.53	51.74	
17-Oct	124	80.02	86.44	90.68		80.63	71.81	70.42			77.83	97.77	86.31	88.62	97.82	85.02	
7-Nov	144	107.22	104.44	99.94		117.08	113.17	130.18			108.27	94.19	105.35	118.78	158.98	191.34	
14-Nov	151	107.91	106.18	111.56		125.70	114.63	124.02			121.43	125.01	125.75	175.29	214.76	184.57	

Group 3	M4C3 G30 Fe10					M1C1 G0 Fe0			M1C1 G10 Fe10				M1C1 G0 Fe10			Sand Spike			Soil Spike		
19-Jun			0.01	0.01																0.01	
26-Jun	0.04	0.04	0.03						0.02	0.02	0.02				0.01						
3-Jul	0.07	0.08	0.07						0.04	0.05	0.04		0.01	0.01	0.01						
10-Jul	0.10	0.10	0.11						0.07	0.06	0.07		0.01	0.01	0.01	0.01	0.01	0.01			
17-Jul	0.13	0.13	0.14						0.07	0.09	0.02		0.02	0.01	0.01						
25-Jul	0.03	0.11		0.09	0.14					0.02	0.05	0.10	0.02	0.02	0.02						
2-Aug	0.14	0.16	0.15						0.12	0.13	0.04			0.01							
8-Aug	0.33	0.33	0.27						0.03	0.16	0.16		0.02	0.02	0.03						
15-Aug	0.31	0.03	0.28	0.31						0.11			0.03	0.02	0.03						
21-Aug		0.26		0.05									0.03	0.03	0.03						
29-Aug	0.53	0.54	0.56				0.01	0.01	0.27	0.23	0.24		0.04	0.03	0.03						
5-Sep		0.56	0.52	0.42		0.01	0.01	0.01	0.23	0.23	0.25		0.04	0.03	0.04						
12-Sep	0.52	0.67	0.57			0.01	0.01	0.01	0.29	0.30	0.26		0.05	0.04	0.04	0.01	0.01	0.01	0.01	0.01	0.01
18-Sep	0.76	0.60	0.66			0.01	0.01	0.01	0.41	0.37	0.34		0.05	0.05	0.10				0.01	0.01	0.01
26-Sep	0.25	0.31		0.06		0.01	0.01	0.01	0.45	0.45	0.44		0.04	0.05	0.05					0.01	
10-Oct	0.61	0.64	0.59			0.01	0.01	0.01	7.41	7.75	7.88		0.06	0.06	0.05	0.01	0.01	0.01			
17-Oct	0.80	0.71	1.70			0.01	0.01	0.01	22.57	21.75	24.22		0.08	0.08	0.07	0.01	0.01	0.01	0.01	0.01	0.01
7-Nov	0.82	0.88	0.94			0.01	0.01	0.01	163.85	163.32	172.21		0.13	0.13	0.13	0.01	0.01	0.01	0.01	0.01	0.01
14-Nov	0.95	0.89	0.87			0.01	0.01	0.01	213.80	217.35	197.96		0.13	0.12	0.12	0.01	0.01	0.01	0.01	0.01	0.01

Appendix A9: Biowall well (BW) field measurements, physical parameters

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW01	10/22/2013	8.56	83.02	6.26	>999	2.2	4.12	16.73	na	na	1.85	-78	136
BW01	11/14/2013	9.06	82.52	6.16	353	2.0	3.84	12.60	16.8	0.75	0.26	-70	144
BW01	11/20/2013	10.85	80.73	6.52	>999	2.2	4.26	12.79	na	0.95	0.74	-118	96
BW01	12/3/2013	8.32	83.26	6.59	189	2.4	4.58	13.90	15.4	0.23	0.28	-68	146
BW01	12/18/2013	6.75	84.83	6.12	45.4	1.3	2.54	7.23	12.3	0.31	0.51	60	277
BW01	1/9/2014	5.98	85.60	5.86	217	0.3	0.603	4.66	12.6	0.14	0.24	128	345
BW01	2/4/2014	5.15	86.43	4.90	281	0.4	0.830	4.11	9.5	0.63	0.37	148	365
BW01	2/17/2014	5.35	86.23	5.26	44.3	0.2	0.416	3.04	7.9	0.54	0.32	127	344
BW01	3/5/2014	5.34	86.24	5.54	255	0.2	0.489	5.48	8.9	na	0.07	-4	213
BW01	3/21/2014	5.41	86.17	5.55	0	0.2	0.407	5.96	9.2	0.20	0.14	117	334
BW01	4/2/2014	5.08	86.50	4.51	95	0.2	0.429	7.25	10.2	1.07	1.88 R	147	364
BW01	4/18/2014	5.15	86.43	6.06	266	0.2	0.319	10.39	10.3	1.23	1.65 R	167	384
BW01	4/28/2014	5.83	85.75	5.42	648	0.2	0.468	15.14	10.9	1.70	2.10 R	128	342
BW01	5/13/2014	5.97	85.61	5.46	63.8	0.1	0.309	24.26	12.0	1.85	2.28 R	109	316
BW01	6/4/2014	6.14	85.44	6.32	359	0.5	1.100	26.01	na	na	2.82 R	-46	161
BW01	6/18/2014	6.61	84.97	6.16	0	0.3	0.614	22.50	12.3	1.27	3.91 R	15	222
BW01	7/2/2014	7.30	84.28	5.88	381	0.2	0.400	26.37	12.9	2.73	3.85 R	53	260
BW01	7/18/2014	7.72	83.86	5.94	160	0.2	0.343	22.65	15.1	4.63	2.86 R	-45	162
BW01	7/29/2014	8.04	83.54	6.00	221	0.2	0.384	21.68	13.3	5.70	3.41 R	-40	170
BW01	8/13/2014	8.18	83.40	6.14	315	0.4	0.896	21.76	13.9	3.66	4.75 R	-54	156
BW01	9/8/2014	8.62	82.96	6.00	160	0.8	1.60	20.59	15.0	4.76	7.37 R	-40	170
BW01	9/24/2014	8.76	82.82	4.74	484	1.0	2.00	18.20	na	na	4.05 R	-39	171
BW01	10/7/2014	9.17	82.41	5.62	193	1.0	2.03	17.23	15.4	7.01	5.65 R	-32	182
BW01	10/21/2014	8.81	82.77	5.81	165	1.0	2.02	17.26	16.5	8.37	6.78 R	-27	187
BW01	11/3/2014	9.00	82.58	5.85	180	1.1	2.19	15.16	16.0	8.17	5.15 R	-77	137
BW01	11/20/2014	8.70	82.88	6.20	245	1.2	2.30	13.52	15.7	9.53	6.25 R	-61	153
BW01	12/2/2014	8.40	83.18	6.02	245	1.2	2.30	14.09	15.5	8.87	6.14 R	-46	168
BW01	12/18/2014	7.60	83.98	5.04	373	1.6	3.12	8.75	na	na	6.36 R	-20	197
BW01	12/30/2014	6.76	84.82	5.94	60.5	0.3	0.602	8.86	13.4	8.70	5.32 R	10	227
BW01	1/13/2015	6.06	85.52	5.66	74.4	0.2	0.382	6.62	11.7	8.00	7.93 R	96	313
BW01	1/28/2015	5.50	86.08	7.41	81.1	0.2	0.386	5.83	12.9	4.26	4.91 R	-16	201
BW01	2/10/2015	6.02	85.56	7.12	73.2	0.1	0.278	5.48	13.1	6.75	4.74 R	-30	187
BW01	3/9/2015	5.05	86.53	5.22	61	0.1	0.320	5.32	12.0	8.12	5.50 R	-74	143
BW01	3/23/2015	5.18	86.40	5.33	271	0.2	0.346	7.21	na	na	5.45 R	-10	207
BW01	4/8/2015	4.85	86.73	7.67	82.5	0.1	0.228	8.04	10.6	4.66	4.36 R	-58	159
BW01	4/24/2015	5.49	86.09	8.22	82.2	0.1	0.273	11.96	10.9	4.85	3.04 R	-53	164
BW01	5/7/2015	6.45	85.13	7.10	119	0.1	0.224	17.60	10.9	0.18	0.00	-42	175
BW01	5/20/2015	7.00	84.58	7.01	113	0.1	0.223	16.87	10.9	0.42	0.00	-56	161
BW01	6/5/2015	6.74	84.84	6.11	171	0.2	0.398	17.02	16.0	0.29	0.98	171	388
BW01	6/24/2015	5.95	85.63	6.02	86.5	0.1	0.278	19.47	na	na	0.02	191	408
BW01	7/7/2015	4.98	86.60	4.73	135	0.3	0.595	23.56	20.0	0.80	1.01 R	238	455
BW01	7/23/2015	7.18	84.40	6.97	136	1.0	0.200	24.09	17.5	1.41	0.75 R	3	220
BW01	8/19/2015	8.28	83.30	4.73	199	0.5	0.957	23.15	15.9	2.15	2.15 R	-23	194
BW01	9/9/2015	9.16	82.42	7.67	317	0.6	1.170	22.55	17.0	2.89	2.43 R	-84	133
BW01	9/29/2015	9.60	81.98	5.42	663	1.0	2.010	19.90	na	na	0.00	-69	148
BW01	10/13/2015	7.88	83.7	5.77	427	1.0	2.090	17.01	15.7	0.00	0.04	-58	159
BW01	11/2/2015	8.31	83.27	5.65	409	0.8	1.620	15.86	16.3	0.46	0.04	-37	180
BW01	11/17/2015	7.84	83.74	5.57	441	0.7	1.390	14.30	16.1	0.45	0.57	-32	185
BW01	12/4/2015	7.36	84.22	na	na	na	na	na	na	0.67	na	na	na
BW01	12/21/2015	8.25	83.33	6.08	429	0.5	1.190	14.05	14.6	0.51	0.68	-45	172
BW01	1/20/2016	5.98	85.6	5.57	482	0.3	0.745	7.69	na	na	0.26	-4	213
BW01	2/5/2016	4.52	87.06	6.89	>999	0.3	0.578	6.35	11.0	0.46	0.38	-8	209
BW01	2/18/2016	5.46	86.12	6.36	474	0.2	0.488	5.21	8.8	0.42	0.72	3	220
BW01	3/7/2016	5.82	85.76	5.73	>999	0.3	0.530	14.37	10.1	0.65	0.56	41	258
BW01	3/22/2016	6.02	85.56	5.53	214	0.2	0.359	9.70	9.2	0.68	0.50	34	251
BW01	4/11/2016	6.29	85.29	5.65	419	0.2	0.349	11.44	na	na	0.01	20	237
BW01	5/24/2016	6.06	85.52	6.99	270	0.1	0.308	17.56	13.9	0.01	0.01	-36	181
BW01	6/8/2016	6.16	85.42	6.29	341	0.1	0.337	16.59	12.4	0.48	0.57	9	226
BW01	6/24/2016	8.75	82.83	6.73	240	0.3	0.701	17.38	na	na	0.01	-20	197
BW01	7/13/2016	7.87	83.71	6.85	176	0.1	0.212	24.15	16.0	0.82	0.67	-9	208
BW01	7/27/2016	8.27	83.31	7.03	861	0.5	0.989	16.91	18.8	1.05	0.01	-51	166
BW01	8/18/2016	8.25	83.33	6.53	>1000	0.5	0.985	22.17	16.4	1.42	0.87	-49	168
BW01	8/31/2016	8.65	82.93	5.72	998	0.7	1.39	21.83	15.8	2.03	1.17	-60	157
BW01	9/22/2016	9.94	81.64	5.80	663	0.7	1.46	18.57	na	na	1.49	0	217

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW02	10/22/2013	7.60	83.23	6.25	>999	1.9	3.32	16.92	na	na	1.30	-77	137
BW02	11/14/2013	8.48	82.35	6.03	583	2.0	3.90	12.48	15.7	0.49	0.21	-81	136
BW02	11/20/2013	8.51	82.32	6.51	259	2.1	3.93	14.17	na	0.61	0.47	-122	92
BW02	12/3/2013	7.25	83.58	6.96	450	2.1	4.07	14.01	15.4	0.14	0.20	-91	123
BW02	12/18/2013	6.58	84.25	6.31	810	1.5	2.84	8.78	14.4	0.19	0.39	-33	184
BW02	1/9/2014	6.98	83.85	6.74	400	0.8	1.69	6.71	12.7	0.30	0.56	80	297
BW02	2/4/2014	5.40	85.43	5.13	168	0.5	1.07	4.16	11.0	na	0.40	56	273
BW02	2/17/2014	5.81	85.02	6.18	205	0.9	1.86	4.38	10.2	na	0.37	5	222
BW02	3/5/2014	5.62	85.21	5.77	32.6	0.8	1.57	4.17	9.5	na	0.25	-3	214
BW02	3/21/2014	5.52	85.31	5.93	134	0.8	1.58	7.37	9.2	na	0.63	94	311
BW02	4/2/2014	5.40	85.43	5.59	173	0.8	1.53	8.59	9.9	na	1.50 R	7	224
BW02	4/18/2014	5.36	85.47	6.88	117	0.7	1.50	10.66	9.8	na	1.07 R	22	239
BW02	4/28/2014	5.76	85.07	6.47	241	0.9	1.80	11.12	11.2	na	1.35 R	-13	204
BW02	5/13/2014	5.76	85.07	6.28	260	0.8	1.58	19.09	11.8	na	2.05 R	-19	191
BW02	6/4/2014	5.81	85.02	6.58	95.6	0.9	1.72	20.63	na	na	3.49 R	-69	141
BW02	6/18/2014	6.25	84.58	6.60	355	1.0	1.95	22.35	12.6	na	2.02 R	-56	154
BW02	7/2/2014	6.85	83.98	6.65	515	1.0	2.02	22.85	13.6	na	3.06 R	-78	129
BW02	7/18/2014	6.93	83.90	6.39	459	1.2	2.28	20.76	14.2	na	3.02 R	-88	122
BW02	7/29/2014	7.86	82.97	6.47	532	1.2	2.25	19.15	14.0	na	3.93 R	-84	126
BW02	8/13/2014	6.71	84.12	6.42	423	1.1	2.10	21.87	13.3	na	4.78 R	-69	141
BW02	9/8/2014	7.54	83.29	6.04	295	1.3	2.44	20.05	15.6	na	6.86 R	-53	157
BW02	9/24/2014	8.20	82.63	4.93	386	1.4	2.76	17.65	na	na	4.38 R	-56	154
BW02	10/7/2014	8.53	82.30	5.68	281	1.4	2.67	17.11	15.9	na	5.80 R	-38	176
BW02	10/21/2014	7.81	83.02	5.81	202	1.3	2.54	17.28	16.6	na	6.93 R	-30	184
BW02	11/3/2014	8.18	82.65	5.89	248	1.3	2.54	15.18	16.1	na	5.15 R	-79	135
BW02	11/20/2014	7.56	83.27	6.20	254	1.3	2.52	13.16	15.8	na	6.27 R	-61	153
BW02	12/2/2014	7.22	83.61	6.05	312	1.0	1.96	13.84	15.5	na	6.20 R	-46	168
BW02	12/18/2014	6.75	84.08	4.66	257	0.7	1.45	10.40	na	na	5.96 R	12	229
BW02	12/30/2014	6.67	84.16	6.20	260	0.7	1.38	9.59	13.9	na	5.49 R	-11	206
BW02	1/13/2015	5.45	85.38	5.79	116	0.3	0.63	5.46	12.6	na	7.05 R	72	289
BW02	1/28/2015	5.94	84.89	7.27	381	0.5	1.05	6.92	12.5	na	4.99 R	-19	198
BW02	2/10/2015	6.26	84.57	6.63	243	0.6	1.45	6.74	13.1	na	4.87 R	-12	205
BW02	3/9/2015	5.49	85.34	5.02	169	0.4	0.929	7.17	10.7	na	5.62 R	-67	150
BW02	3/23/2015	5.89	84.94	5.60	161	0.5	1.12	10.46	na	na	5.61 R	-45	172
BW02	4/8/2015	5.22	85.61	7.25	171	0.6	1.15	7.87	10.2	na	4.79 R	-48	169
BW02	4/24/2015	5.79	85.04	7.70	187	0.8	1.57	12.46	10.1	na	2.99 R	-30	187
BW02	5/7/2015	6.25	84.58	7.12	245	0.9	1.69	16.54	11.3	na	0.00	-56	161
BW02	5/20/2015	6.70	84.13	6.65	251	0.9	1.70	15.61	11.1	na	0.00	-53	164
BW02	6/5/2015	6.10	84.73	6.95	194	1.0	1.92	17.86	12.2	na	0.17	-47	170
BW02	6/24/2015	5.95	84.88	6.58	209	1.0	1.88	23.40	na	na	0.08	135	352
BW02	7/7/2015	5.45	85.38	5.70	259	0.1	0.190	23.97	16.5	na	1.41 R	126	343
BW02	7/23/2015	6.98	83.85	7.24	205	1.2	2.29	22.89	16.7	na	1.28 R	-8	209
BW02	8/19/2015	8.16	82.67	5.07	275	2.3	4.290	23.68	14.6	na	2.54 R	-57	160
BW02	9/9/2015	8.74	82.09	7.74	306	1.1	2.070	22.59	16.6	na	2.70 R	-104	113
BW02	9/29/2015	9.57	81.26	5.52	411	1.4	2.650	18.10	na	na	0.00	-87	130
BW02	10/13/2015	6.95	83.88	5.92	462	1.2	2.910	17.04	15.80	na	0.08	-94	123
BW02	11/2/2015	7.68	83.15	5.61	343	1.0	1.930	15.89	16.90	na	0.08	-34	183
BW02	11/17/2015	7.35	83.48	5.68	379	0.7	1.480	14.33	16.30	na	0.51	-47	170
BW02	12/4/2015	6.64	84.19	6.10	478	0.5	1.090	13.00	15.40	na	0.51	-30	187
BW02	12/21/2015	7.65	83.18	6.29	513	0.7	1.150	13.87	15.10	na	0.66	-41	176
BW02	1/20/2016	6.58	84.25	5.66	482	0.7	1.540	7.19	na	na	0.07	-21	196
BW02	2/5/2016	5.31	85.52	6.24	>999	0.9	1.89	6.23	10.2	na	0.41	-1	216
BW02	2/18/2016	5.95	84.88	6.02	508	0.8	1.58	6.24	9.4	na	0.19	-41	176
BW02	3/7/2016	6.09	84.74	5.95	>999	0.5	1.04	13.29	11.1	na	0.19	-61	156
BW02	3/22/2016	6.29	84.54	5.44	493	0.8	1.50	11.05	10.2	na	0.47	-67	150
BW02	4/8/2016	6.11	84.72	7.43	547	1.2	2.36	12.19	na	na	0.01	-105	112
BW02	5/24/2016	5.88	84.95	6.89	300	0.7	1.46	18.37	13.1	na	0.01	-51	166
BW02	6/8/2016	6.06	84.77	6.93	204	0.5	1.05	17.07	11.5	na	0.11	-67	150
BW02	6/24/2016	8.15	82.68	7.29	325	0.5	0.95	16.08	na	na	0.01	-50	167
BW02	7/13/2016	7.61	83.22	7.04	223	1.2	2.19	22.94	15.7	na	0.51	6	223
BW02	7/27/2016	8.25	82.58	7.08	833	0.6	1.15	17.43	15.1	na	0.01	-58	159
BW02	8/18/2016	7.17	83.66	6.82	>1000	0.4	0.78	22.59	16.4	na	0.86	-65	152
BW02	8/31/2016	8.30	82.53	5.96	>1000	0.6	1.15	20.87	16.7	na	1.68	-66	151
BW02	9/22/2016	9.42	81.41	6.07	751	0.8	1.59	19.25	na	na	1.29	-21	196

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW03	10/22/2013	6.85	83.52	6.15	>999	2.3	4.39	16.50	na	na	1.15	-81	133
BW03	11/14/2013	8.04	82.33	6.08	110	2.4	4.62	12.75	15.6	0.40	0.23	-70	144
BW03	11/20/2013	8.01	82.36	6.60	59.6	2.4	4.52	12.51	na	0.52	0.43	-124	90
BW03	12/3/2013	6.45	83.92	6.90	299	2.4	4.49	13.99	14.8	0.10	0.35	-92	122
BW03	12/18/2013	5.89	84.48	6.41	540	2.5	4.70	9.85	14.3	0.24	0.36	-61	156
BW03	1/9/2014	5.79	84.58	6.89	465	2.5	4.73	8.48	13.3	0.32	0.68	35	252
BW03	2/4/2014	5.59	84.78	5.93	658	2.5	4.73	7.27	12.5	na	0.42	-15	202
BW03	2/17/2014	5.70	84.67	6.42	4.69	2.4	4.69	6.48	11.2	na	0.35	-42	175
BW03	3/5/2014	5.46	84.91	5.94	225	2.1	4.91	2.37	9.8	na	0.06	-19	198
BW03	3/21/2014	5.20	85.17	5.99	354	2.6	4.98	7.90	10.8	na	0.56	42	259
BW03	4/2/2014	5.01	85.36	5.41	391	2.6	5.02	8.30	10.6	na	1.65 R	14	231
BW03	4/18/2014	5.01	85.36	6.69	203	2.4	4.57	10.53	10.6	na	1.29 R	36	253
BW03	4/28/2014	5.34	85.03	6.34	274	2.5	4.66	11.06	10.9	na	0.97 R	-1	216
BW03	5/13/2014	5.31	85.06	6.27	256	2.2	4.08	17.19	11.4	na	1.82 R	-15	199
BW03	6/4/2014	5.29	85.08	6.70	261	2.7	4.97	19.53	na	na	3.58 R	-73	137
BW03	6/18/2014	5.60	84.77	6.49	402	2.4	4.59	20.02	12.4	na	1.80 R	-51	159
BW03	7/2/2014	6.13	84.24	6.59	349	2.3	4.34	20.60	12.8	na	2.93 R	-72	138
BW03	7/18/2014	6.42	83.95	6.41	332	2.3	4.27	16.93	12.9	na	2.92 R	-78	136
BW03	7/29/2014	7.10	83.27	6.42	522	2.1	4.05	17.59	13.1	na	4.15 R	-75	139
BW03	8/13/2014	6.61	83.76	6.25	610	2.2	4.12	20.30	13.9	na	5.11 R	-60	150
BW03	9/8/2014	7.08	83.29	6.12	375	2.2	4.08	18.34	14.2	na	6.24 R	-50	160
BW03	9/24/2014	7.50	82.87	5.07	>999	2.3	4.34	16.20	na	na	5.20 R	-64	150
BW03	10/7/2014	7.87	82.50	5.56	257	2.2	4.11	16.03	14.6	na	6.01 R	-26	188
BW03	10/21/2014	7.15	83.22	5.68	283	1.9	3.57	16.46	15.7	na	7.20 R	-17	197
BW03	11/3/2014	7.53	82.84	5.75	399	1.7	3.28	14.90	14.9	na	5.06 R	-73	141
BW03	11/20/2014	6.75	83.62	6.02	340	1.8	3.41	13.17	15.0	na	6.30 R	-47	167
BW03	12/2/2014	6.42	83.95	5.95	383	1.6	3.09	14.22	15.0	na	6.34 R	-42	172
BW03	12/18/2014	5.83	84.54	5.01	386	1.4	2.81	9.64	na	na	6.85 R	0	217
BW03	12/30/2014	5.91	84.46	6.07	397	1.6	3.11	10.67	14.1	na	5.56 R	-12	205
BW03	1/13/2015	5.59	84.78	6.05	343	1.6	3.15	8.19	13.9	na	6.48 R	22	239
BW03	1/28/2015	5.63	84.74	6.87	419	1.9	3.75	8.56	13.4	na	5.08 R	-11	206
BW03	2/10/2015	5.82	84.55	6.19	375	2.0	3.92	8.10	13.0	na	5.06 R	2	219
BW03	3/9/2015	5.21	85.16	4.55	436	2.0	3.91	7.81	11.6	na	5.83 R	-52	165
BW03	3/23/2015	5.54	84.83	5.76	669	2.2	4.29	9.72	na	na	7.05 R	-63	154
BW03	4/8/2015	5.46	84.91	6.76	339	1.8	3.57	7.93	10.4	na	4.87 R	-30	187
BW03	4/24/2015	5.35	85.02	7.30	256	1.9	3.65	11.07	11.8	na	2.99 R	-39	178
BW03	5/7/2015	5.65	84.72	6.70	284	1.8	3.49	14.99	11.2	na	0.00	-44	173
BW03	5/20/2015	5.69	84.68	6.25	363	1.6	3.13	12.19	11.2	na	0.00	-38	179
BW03	6/5/2015	5.40	84.97	6.70	296	1.6	3.16	15.46	11.8	na	0.07	-53	164
BW03	6/24/2015	6.10	84.27	6.71	455	1.9	3.50	22.51	na	na	0.13	141	358
BW03	7/7/2015	5.02	85.35	4.68	320	4.8	8.79	21.49	14.7	na	1.38 R	156	373
BW03	7/23/2015	6.22	84.15	6.79	238	1.9	3.58	19.60	14.3	na	1.65 R	26	243
BW03	8/19/2015	7.43	82.94	4.47	290	4.0	7.28	19.62	14.5	na	2.72 R	-26	191
BW03	9/9/2015	7.97	82.40	7.43	404	0.8	3.18	20.41	15.3	na	3.67 R	-84	133
BW03	9/29/2015	8.18	82.19	5.41	853	1.8	3.36	19.29	na	na	0.00	-89	128
BW03	10/13/2015	6.62	83.75	5.51	823	1.6	3.28	17.31	16.3	na	0.00	-87	130
BW03	11/2/2015	6.76	83.61	5.39	470	1.6	3.09	15.61	16.1	na	0.01	-47	170
BW03	11/17/2015	6.46	83.91	5.53	477	1.0	2.07	14.18	15.4	na	0.48	-50	167
BW03	12/4/2015	5.97	84.40	6.07	712	1.1	2.22	12.63	15.0	na	0.73	-58	159
BW03	12/21/2015	6.74	83.63	6.24	722	1.1	2.30	13.09	14.5	na	0.78	-64	153
BW03	1/15/2016	5.61	84.76	6.40	878	2.2	4.21	10.50	na	na	0.01	-55	162
BW03	2/5/2016	4.98	85.39	6.48	>999	1.4	2.74	6.49	11.2	na	0.33	-47	170
BW03	2/18/2016	6.67	83.70	5.36	565	0.7	1.45	7.64	11.7	na	0.23	-28	189
BW03	3/7/2016	5.66	84.71	5.40	101	0.6	1.25	11.49	12.4	na	0.17	-53	164
BW03	3/22/2016	5.78	84.59	4.88	554	1.6	3.20	10.79	10.9	na	0.45	-58	159
BW03	4/8/2016	5.46	84.91	7.00	788	2.7	5.00	13.16	na	na	0.01	-111	106
BW03	5/24/2016	5.38	84.99	7.08	313	1.9	3.65	15.39	12.3	na	0.01	-102	115
BW03	6/8/2016	5.51	84.86	6.98	213	1.4	2.74	16.19	12.9	na	0.01	-117	100
BW03	6/24/2016	7.61	82.76	7.35	583	1.1	2.22	19.01	na	na	0.01	-70	147
BW03	7/13/2016	6.78	83.59	6.59	239	1.9	3.56	20.15	14.7	na	0.65	26	243
BW03	7/27/2016	7.32	83.05	6.71	888	1.3	2.61	17.28	13.8	na	0.01	-54	163
BW03	8/18/2016	5.96	84.41	6.60	>1000	1.1	2.17	20.84	14.8	na	0.75	-84	133
BW03	8/31/2016	7.27	83.10	5.77	>1000	1.2	2.26	19.60	14.9	na	1.43	-82	135
BW03	9/22/2016	8.97	81.40	5.82	714	1.6	2.98	18.90	na	na	1.64	-33	184

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW04	10/22/2013	6.51	83.58	6.19	>999	1.4	2.78	16.30	na	na	1.13	-59	155
BW04	11/14/2013	7.29	82.80	5.80	373	1.2	2.31	12.47	15.9	0.41	0.23	-31	186
BW04	11/20/2013	7.55	82.54	6.48	82.9	1.8	3.38	12.71	na	0.50	0.43	-88	126
BW04	12/3/2013	5.98	84.11	6.95	152	1.0	2.04	13.24	14.8	0.20	0.35	-72	142
BW04	12/18/2013	5.45	84.64	6.38	422	1.0	2.07	10.05	14.1	0.15	0.33	-50	167
BW04	1/9/2014	5.44	84.65	6.95	103	0.9	1.83	7.97	13.7	0.30	0.48	8	225
BW04	2/4/2014	5.19	84.90	6.06	343	1.1	2.26	7.05	12.6	na	0.34	-3	214
BW04	2/17/2014	5.26	84.83	6.76	190	1.0	2.01	5.81	11.0	na	0.44	-36	181
BW04	3/5/2014	5.09	85.00	5.79	68.5	1.3	6.63	3.10	9.1	na	0.05	48	265
BW04	3/21/2014	4.72	85.37	6.23	282	1.1	2.18	7.36	10.6	na	0.54	21	238
BW04	4/2/2014	4.55	85.54	5.51	324	1.2	2.30	8.04	10.6	na	1.75 R	18	235
BW04	4/18/2014	4.57	85.52	6.79	202	1.0	2.03	11.00	10.6	na	1.04 R	38	255
BW04	4/28/2014	4.93	85.16	6.39	181	1.1	2.17	11.50	11.7	na	1.05 R	5	222
BW04	5/13/2014	4.93	85.16	6.26	193	0.9	1.86	17.22	11.4	na	2.07 R	1	215
BW04	6/4/2014	4.84	85.25	4.44	186	0.9	1.85	17.87	na	na	4.04 R	-39	171
BW04	6/18/2014	5.21	84.88	6.59	256	0.9	1.82	19.33	12.3	na	1.86 R	-46	164
BW04	7/2/2014	5.78	84.31	6.55	242	0.9	1.73	20.60	12.6	na	2.91 R	-56	154
BW04	7/18/2014	6.09	84.00	6.36	217	0.9	1.86	17.19	13.1	na	3.24 R	-70	144
BW04	7/29/2014	6.84	83.25	6.43	256	0.8	1.63	17.50	13.4	na	4.31 R	-65	145
BW04	8/13/2014	6.70	83.39	6.38	237	0.9	1.78	20.50	13.7	na	4.60 R	-68	142
BW04	9/8/2014	6.84	83.25	6.16	176	0.9	1.81	17.89	14.9	na	5.68 R	-36	174
BW04	9/24/2014	7.37	82.72	4.95	978	1.5	2.96	15.62	na	na	5.79 R	-56	158
BW04	10/7/2014	7.62	82.47	5.67	163	1.0	2.05	15.99	14.7	na	6.12 R	-29	185
BW04	10/21/2014	7.00	83.09	5.81	142	0.9	1.80	16.05	14.6	na	7.45 R	-21	193
BW04	11/3/2014	7.14	82.95	5.90	175	0.8	1.68	14.70	14.5	na	4.92 R	-75	139
BW04	11/20/2014	6.38	83.71	6.19	198	0.8	1.67	12.50	15.0	na	6.08 R	-55	159
BW04	12/2/2014	5.89	84.20	6.11	195	0.7	1.49	13.95	14.7	na	6.43 R	-44	170
BW04	12/18/2014	5.33	84.76	4.80	312	0.8	1.59	10.25	na	na	5.55 R	-2	215
BW04	12/30/2014	5.35	84.74	6.30	166	0.7	1.32	9.76	13.6	na	5.75 R	-16	201
BW04	1/13/2015	5.14	84.95	6.41	218	0.6	1.28	8.29	13.6	na	6.19 R	6	223
BW04	1/28/2015	4.96	85.13	7.27	256	0.7	1.38	7.79	12.6	na	5.22 R	-19	198
BW04	2/10/2015	5.24	84.85	6.68	271	0.5	1.13	7.65	12.9	na	12.90 R	-9	208
BW04	3/9/2015	4.61	85.48	5.04	268	0.5	1.10	7.44	10.7	na	5.97 R	-65	152
BW04	3/23/2015	4.82	85.27	5.55	297	0.4	0.803	7.58	na	na	7.96 R	-16	201
BW04	4/8/2015	4.80	85.29	7.26	253	0.5	0.944	7.84	10.2	na	5.07 R	-41	176
BW04	4/24/2015	4.63	85.46	7.75	228	0.5	0.986	11.10	11.3	na	3.04 R	-42	175
BW04	5/7/2015	5.09	85.00	7.13	261	0.4	0.879	13.71	11.2	na	0.00	-41	176
BW04	5/20/2015	5.30	84.79	6.78	337	0.4	0.881	12.29	11.1	na	0.13	-43	174
BW04	6/5/2015	4.99	85.10	7.30	202	0.5	1.030	15.16	11.7	na	0.23	-65	152
BW04	6/24/2015	5.40	84.69	6.58	229	0.4	0.836	19.64	na	na	0.09	160	377
BW04	7/7/2015	4.45	85.64	5.18	281	1.3	2.600	22.19	14.0	na	1.34 R	158	375
BW04	7/23/2015	5.72	84.37	7.72	230	0.4	0.766	19.75	14.4	na	1.67 R	13	230
BW04	8/19/2015	6.78	83.31	4.98	276	0.9	1.710	19.71	14.3	na	1.71 R	-25	192
BW04	9/9/2015	7.59	82.50	7.85	298	0.5	0.963	20.55	15.4	na	3.39 R	-86	131
BW04	9/29/2015	8.08	82.01	5.24	675	0.5	1.040	19.62	na	na	0.05	-42	175
BW04	10/13/2015	5.85	84.24	5.89	712	0.8	1.310	16.12	15.2	na	0.02	-48	169
BW04	11/2/2015	6.38	83.71	5.70	344	0.7	1.470	15.12	15.5	na	0.10	-40	177
BW04	11/17/2015	5.97	84.12	5.79	380	0.5	1.070	13.83	15.3	na	0.43	-64	153
BW04	12/4/2015	5.44	84.65	6.07	>999	0.6	1.220	12.73	14.6	na	0.74	-33	184
BW04	12/21/2015	6.37	83.72	6.22	>999	0.5	1.310	12.84	14.1	na	0.78	-36	181
BW04	1/15/2016	5.22	84.87	6.74	674	0.6	1.180	10.50	na	na	0.08	-31	186
BW04	2/5/2016	4.40	85.69	6.66	>999	0.7	2.120	6.51	10.8	na	0.48	-34	183
BW04	2/18/2016	5.06	85.03	5.92	609	0.5	1.010	6.80	11.0	na	0.40	-2	215
BW04	3/7/2016	5.04	85.05	5.90	>999	0.4	0.728	11.10	12.2	na	0.22	-47	170
BW04	3/22/2016	5.14	84.95	5.44	362	0.4	0.812	11.15	10.9	na	0.37	-51	166
BW04	4/8/2016	4.86	85.23	7.40	479	0.4	0.841	12.52	na	na	0.68	-84	133
BW04	5/24/2016	4.72	85.37	7.70	214	0.6	1.13	16.84	12.3	na	0.19	-106	111
BW04	6/8/2016	4.82	85.27	7.09	391	0.4	0.806	16.53	11.6	na	0.63	-81	136
BW04	6/24/2016	7.35	82.74	7.05	489	0.3	0.665	18.20	na	na	0.01	-42	175
BW04	7/13/2016	6.21	83.88	7.23	265	0.4	0.74	20.06	15.1	na	0.67	21	238
BW04	7/27/2016	6.76	83.33	7.09	973	0.4	0.89	17.18	14.2	na	0.01	-55	162
BW04	8/18/2016	6.51	83.58	6.94	933	0.5	1.03	19.86	16.3	na	0.87	-76	141
BW04	8/31/2016	7.76	82.33	6.12	867	0.6	1.18	18.70	15.1	na	1.71	-77	140
BW04	9/22/2016	8.56	81.53	6.01	868	0.6	1.17	17.71	na	na	1.89	2	219

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW05	10/22/2013	8.13	82.60	6.40	>999	2.2	4.21	15.79	na	na	1.38	-78	136
BW05	11/14/2013	7.78	82.95	6.22	89.8	2.4	4.62	12.76	15.1	0.32	0.25	-67	147
BW05	11/20/2013	8.35	82.38	6.59	113	2.5	4.74	11.72	na	0.47	0.47	-107	110
BW05	12/3/2013	6.42	84.31	6.76	122	2.3	4.35	13.17	14.7	0.22	0.32	-67	147
BW05	12/18/2013	5.80	84.93	6.37	547	2.3	4.43	9.82	13.7	0.14	0.30	-66	151
BW05	1/9/2014	5.86	84.87	7.06	358	2.4	4.57	8.39	13.2	0.35	0.50	-23	194
BW05	2/4/2014	5.55	85.18	6.10	387	1.8	3.54	9.57	12.7	na	0.26	-31	186
BW05	2/17/2014	5.74	84.99	6.58	217	2.2	4.33	5.65	11.0	na	0.39	-50	167
BW05	3/5/2014	5.38	85.35	6.76	351	2.3	4.49	8.03	10.4	na	0.04	-91	126
BW05	3/21/2014	5.25	85.48	6.07	361	2.4	4.63	7.37	10.9	na	0.78	19	236
BW05	4/2/2014	5.19	85.54	5.40	638	2.5	4.73	7.78	10.1	na	1.70 R	15	232
BW05	4/18/2014	5.10	85.63	7.08	276	1.6	3.02	11.19	10.5	na	1.22 R	42	259
BW05	4/28/2014	5.46	85.27	6.36	337	2.3	4.46	11.47	10.9	na	1.02 R	5	222
BW05	5/13/2014	5.48	85.25	6.27	412	2.1	3.92	16.95	11.4	na	2.35 R	8	222
BW05	6/4/2014	5.40	85.33	6.62	202	2.4	4.47	19.31	na	na	3.87 R	-48	162
BW05	6/18/2014	5.77	84.96	6.54	422	2.3	4.41	19.21	12.1	na	1.62 R	-55	155
BW05	7/2/2014	6.30	84.43	6.64	6.36	2.1	4.05	20.63	12.5	na	2.95 R	-66	144
BW05	7/18/2014	6.64	84.09	6.39	502	2.2	4.20	17.02	12.4	na	2.22 R	-81	133
BW05	7/29/2014	7.34	83.39	6.41	537	2.0	3.79	17.39	13.2	na	4.48 R	-76	138
BW05	8/13/2014	7.14	83.59	6.34	494	2.1	3.91	20.16	13.9	na	4.57 R	-73	137
BW05	9/8/2014	7.46	83.27	6.06	407	2.0	3.86	17.29	14.1	na	5.31 R	-39	175
BW05	9/24/2014	8.21	82.52	6.55	865	2.4	4.43	16.72	na	na	1.37 R	-67	147
BW05	10/7/2014	8.18	82.55	5.59	328	2.1	3.96	15.73	14.3	na	6.34 R	-31	183
BW05	10/21/2014	7.61	83.12	5.71	287	1.7	3.63	15.85	15.0	na	7.70 R	-25	189
BW05	11/3/2014	7.66	83.07	5.73	284	1.9	3.66	14.41	14.7	na	4.84 R	-80	134
BW05	11/20/2014	7.01	83.72	6.11	317	1.9	3.60	12.31	14.5	na	6.01 R	-63	154
BW05	12/2/2014	6.40	84.33	5.96	351	1.8	3.43	13.95	14.7	na	6.51 R	-50	164
BW05	12/18/2014	5.94	84.79	5.13	438	2.0	3.90	6.88	na	na	6.50 R	-31	186
BW05	12/30/2014	5.86	84.87	6.15	315	1.8	3.41	9.59	13.7	na	5.97 R	-22	195
BW05	1/13/2015	5.76	84.97	6.20	488	1.6	3.17	8.45	13.3	na	6.01 R	2	219
BW05	1/28/2015	5.62	85.11	6.89	624	1.8	3.52	7.74	12.8	na	5.40 R	-13	204
BW05	2/10/2015	5.85	84.88	6.17	479	1.8	3.57	7.72	12.5	na	5.43 R	5	222
BW05	3/9/2015	5.21	85.52	4.58	391	1.7	3.39	7.71	11.9	na	6.16 R	-55	162
BW05	3/23/2015	5.45	85.28	5.66	350	1.7	3.41	7.30	na	na	8.71 R	-42	175
BW05	4/8/2015	5.44	85.29	6.71	428	2.1	3.28	7.92	11.1	na	5.25 R	-21	196
BW05	4/24/2015	5.25	85.48	7.21	386	1.6	3.12	11.15	11.5	na	3.08 R	-32	185
BW05	5/7/2015	5.73	85.00	6.74	363	1.5	2.87	14.16	11.2	na	0.00	-37	180
BW05	5/20/2015	5.90	84.83	6.39	635	1.3	2.54	12.99	11.0	na	0.00	-35	182
BW05	6/5/2015	5.53	85.20	6.74	320	1.4	2.73	14.27	11.7	na	0.12	-50	167
BW05	6/24/2015	5.50	85.23	6.66	453	1.6	3.06	18.57	na	na	0.07	138	355
BW05	7/7/2015	5.12	85.61	4.46	406	4.3	7.76	21.37	14.0	na	0.43	172	389
BW05	7/23/2015	6.48	84.25	7.13	353	1.4	2.71	21.19	13.7	na	1.88 R	5	222
BW05	8/19/2015	7.68	83.05	4.72	454	2.5	4.62	21.37	13.9	na	3.03 R	-20	197
BW05	9/9/2015	8.22	82.51	7.71	551	1.2	2.29	21.21	15.3	na	3.30 R	-93	124
BW05	9/29/2015	7.79	82.94	5.32	>999	1.3	2.60	17.86	na	na	0.00	-68	149
BW05	10/13/2015	6.58	84.15	5.36	>999	1.3	2.74	17.83	16.1	na	0.00	-72	145
BW05	11/2/2015	6.67	84.06	5.33	542	1.5	3.11	15.42	15.0	na	0.00	-35	182
BW05	11/17/2015	6.42	84.31	5.45	594	1.3	2.56	13.92	14.9	na	0.54	-62	155
BW05	12/4/2015	5.98	84.75	5.89	674	1.4	2.69	12.58	14.6	na	0.50	-56	161
BW05	12/21/2015	6.64	84.09	5.84	609	1.5	2.68	12.98	14.3	na	0.59	-51	166
BW05	1/15/2016	5.81	84.92	6.51	895	2.3	4.44	10.05	na	na	0.01	-63	154
BW05	2/5/2016	4.93	85.80	6.49	>999	2.2	4.22	6.38	11.1	na	0.39	-54	163
BW05	2/18/2016	5.56	85.17	6.59	455	1.5	2.98	5.77	11.3	na	0.35	-55	162
BW05	3/7/2016	5.63	85.10	5.31	455	0.6	1.21	11.56	11.9	na	0.18	-50	167
BW05	3/22/2016	5.75	84.98	4.93	664	0.6	1.27	11.98	10.5	na	0.51	-37	180
BW05	4/8/2016	6.04	84.69	7.10	677	1.8	3.39	11.31	na	na	0.01	-97	120
BW05	5/24/2016	5.28	85.45	7.11	344	1.9	3.61	16.64	12.2	na	0.01	-99	118
BW05	6/8/2016	5.35	85.38	6.79	247	1.4	2.89	17.43	12.1	na	0.36	-73	144
BW05	6/24/2016	6.89	83.84	7.59	>999	1.5	2.93	14.07	na	na	0.01	-90	127
BW05	7/13/2016	6.85	83.88	6.92	364	1.4	2.64	21.61	13.9	na	0.88	24	241
BW05	7/27/2016	7.38	83.35	6.64	919	1.3	2.48	17.56	13.4	na	0.01	-46	171
BW05	8/18/2016	6.90	83.83	6.74	>1000	1.4	2.63	19.45	15.0	na	0.79	-97	120
BW05	8/31/2016	7.30	83.43	5.86	941	1.4	2.67	18.67	14.5	na	1.51	-95	122
BW05	9/22/2016	8.80	81.93	6.03	868	1.5	2.92	16.40	na	na	2.28	-26	191

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW06	10/22/2013	6.82	83.85	5.72	>999	0.8	1.69	15.13	na	na	1.56	-35	179
BW06	11/14/2013	7.56	83.11	5.88	460	1.5	2.96	11.87	15.1	0.26	0.55	-45	172
BW06	11/21/2013	7.44	83.23	6.16	109	0.9	1.88	12.04	na	0.75	0.59	-31	186
BW06	12/3/2013	6.10	84.57	6.19	0	0.8	1.58	12.72	14.5	0.30	0.94	-7	207
BW06	12/18/2013	5.48	85.19	6.16	185	1.0	2.02	8.06	13.5	0.12	0.82	10	227
BW06	1/9/2014	5.60	85.07	6.78	1000	1.3	2.48	8.61	12.5	0.44	1.84	35	252
BW06	2/4/2014	5.15	85.52	6.26	94.4	1.4	2.78	7.43	11.4	0.46	2.20	-26	191
BW06	2/17/2014	5.33	85.34	5.93	67.3	1.4	2.70	6.94	9.8	0.48	1.93	15	232
BW06	3/5/2014	5.08	85.59	6.55	121	1.6	3.07	7.76	10.5	na	0.07	-72	145
BW06	3/21/2014	4.98	85.69	5.97	>999	1.4	2.73	8.69	10.0	0.48	1.38	74	291
BW06	4/2/2014	4.92	85.75	4.97	122	1.4	2.70	8.16	9.4	0.90	3.46 R	88	305
BW06	4/18/2014	4.94	85.73	6.39	>999	1.3	2.56	9.98	10.0	1.30	4.58 R	145	362
BW06	4/28/2014	5.41	85.26	6.14	105	1.3	2.52	12.72	12.7	1.35	3.72 R	45	259
BW06	5/13/2014	5.58	85.09	6.05	49.3	1.1	2.21	19.74	11.2	1.39	4.07 R	22	232
BW06	6/4/2014	5.51	85.16	6.56	133	1.3	2.50	22.87	na	na	1.90 R	-76	131
BW06	6/18/2014	5.90	84.77	6.47	901	1.1	2.10	18.92	12.5	1.20	3.68 R	-25	185
BW06	7/2/2014	6.53	84.14	6.44	90.7	1.0	1.99	21.88	13.2	2.87	4.80 R	-10	200
BW06	7/18/2014	6.67	84.00	6.34	129	1.0	2.03	18.51	13.0	3.85	3.31 R	-68	142
BW06	7/29/2014	7.46	83.21	6.44	200	1.0	2.04	18.90	13.2	na	4.32 R	-74	136
BW06	8/13/2014	6.76	83.91	6.25	0	1.2	2.31	21.26	14.3	3.68	6.39 R	-58	152
BW06	9/8/2014	7.41	83.26	6.17	84.4	1.1	2.23	17.89	14.1	5.71	4.94 R	-45	165
BW06	9/24/2014	8.22	82.45	6.39	176	1.4	2.80	15.93	na	na	1.22 R	-53	161
BW06	10/7/2014	8.16	82.51	5.63	112	1.2	2.32	16.45	15.1	6.63	6.46 R	-27	187
BW06	10/21/2014	7.52	83.15	5.79	89.7	1.1	2.08	16.05	15.3	7.92	8.07 R	-17	197
BW06	11/3/2014	7.51	83.16	5.88	116	1.1	2.16	14.59	14.9	7.77	4.56 R	-74	140
BW06	11/20/2014	6.65	84.02	6.14	120	1.1	2.09	11.93	14.8	9.16	5.77 R	-51	166
BW06	12/2/2014	6.06	84.61	5.99	98.8	0.9	1.79	13.34	14.7	8.58	6.59 R	-36	178
BW06	12/18/2014	5.53	85.14	4.96	248	1.3	2.63	8.95	na	na	7.51 R	-16	201
BW06	12/30/2014	5.63	85.04	6.19	73.6	1.0	2.04	8.82	13.6	7.78	6.20 R	-13	204
BW06	1/13/2015	5.19	85.48	5.92	301	1.0	1.98	8.41	12.3	6.02	5.85 R	37	254
BW06	1/28/2015	5.38	85.29	5.52	97.1	1.0	2.06	5.52	12.4	4.01	5.67 R	-1	216
BW06	2/10/2015	5.75	84.92	6.36	87	1.0	1.92	5.65	12.2	6.01	5.75 R	11	228
BW06	3/9/2015	4.90	85.77	4.75	75.8	0.9	1.79	6.04	10.9	7.13	6.56 R	-44	173
BW06	3/23/2015	5.30	85.37	5.35	178	1.0	2.06	7.95	na	na	4.32 R	-48	169
BW06	4/8/2015	4.92	85.75	6.93	99.5	0.8	1.65	7.63	10.4	4.50	5.51 R	-15	202
BW06	4/24/2015	5.14	85.53	7.38	386	0.8	3.12	11.69	11.7	4.69	3.11 R	-20	197
BW06	5/7/2015	5.79	84.88	6.96	117	0.7	1.44	15.20	10.6	0.13	0.64	-32	185
BW06	5/20/2015	5.89	84.78	6.50	114	0.7	1.32	14.12	10.8	0.78	0.64	-27	190
BW06	6/5/2015	5.21	85.46	6.50	595	0.8	1.58	15.08	11.6	0.36	1.14	54	271
BW06	6/24/2015	5.45	85.22	6.69	138	0.9	1.81	15.07	na	na	0.00	151	368
BW06	7/7/2015	4.90	85.77	4.49	197	1.8	3.44	24.22	13.5	0.48	1.48 R	278	495
BW06	7/23/2015	6.78	83.89	6.89	162	0.7	1.39	18.01	14.1	1.34	2.13 R	93	310
BW06	8/19/2015	7.77	82.90	4.42	143	1.6	3.07	21.07	15.4	1.49	3.00 R	22	239
BW06	9/9/2015	8.16	82.51	7.26	175	0.8	1.55	23.16	15.7	3.11	4.55 R	-59	158
BW06	9/28/2015	8.52	82.15	6.05	>999	0.9	1.82	18.08	na	na	3.82 R	-46	171
BW06	10/13/2015	5.99	84.68	5.22	>999	0.8	1.64	16.20	15.3	0.00	0.16	37	254
BW06	11/2/2015	6.67	84.00	5.13	241	0.8	1.68	16.13	15.5	0.48	0.55	55	272
BW06	11/17/2015	6.24	84.43	5.62	305	0.7	1.39	13.66	14.9	0.19	0.68	-51	166
BW06	12/4/2015	5.52	85.15	6.29	660	0.8	1.53	12.92	14.5	0.98	0.74	-32	185
BW06	12/21/2015	6.63	84.04	6.32	468	0.7	1.54	12.97	13.8	0.67	0.81	-42	175
BW06	1/15/2016	5.84	84.83	6.63	502	1.3	2.64	10.94	na	na	0.04	-35	182
BW06	2/5/2016	4.74	85.93	6.22	>1000	1.1	2.17	8.33	11.8	0.23	0.72	19	236
BW06	2/18/2016	5.38	85.29	5.66	455	0.9	1.73	7.15	12.0	0.34	0.80	51	268
BW06	3/7/2016	5.59	85.08	5.07	609	0.5	0.959	9.17	11.3	na	0.49	80	297
BW06	3/22/2016	5.56	85.11	5.13	245	0.6	1.20	10.17	10.2	0.32	0.37	37	254
BW06	4/8/2016	5.29	85.38	7.16	604	1.3	2.650	10.06	na	na	0.27	-86	131
BW06	5/24/2016	5.10	85.57	7.55	508	0.9	1.72	20.22	12.0	na	0.53	13	230
BW06	6/8/2016	5.27	85.40	6.19	425	0.7	1.37	17.21	12.2	0.42	0.51	27	244
BW06	6/24/2016	8.25	82.42	7.42	505	0.9	1.73	13.04	na	na	0.01	-66	151
BW06	7/13/2016	6.76	83.91	7.02	361	1.5	2.79	21.23	14.6	0.94	0.48	17	234
BW06	7/27/2016	7.60	83.07	6.54	439	0.7	1.43	20.99	14.4	1.30	0.01	81	298
BW06	8/18/2016	6.39	84.28	5.69	>1000	0.7	1.42	18.69	18.8	0.91	1.51	50	267
BW06	8/31/2016	7.53	83.14	5.16	>1000	0.7	1.42	19.78	16.9	1.51	2.04	-34	183
BW06	9/22/2016	9.19	81.48	6.79	621	1.0	1.91	16.45	na	na	2.99	-4	213

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW07	10/22/2013	7.33	83.90	6.04	>999	1.8	3.43	15.74	na	na	1.16	-67	147
BW07	11/14/2013	8.04	83.19	6.04	>999	2.0	3.84	12.54	15.7	0.26	0.20	-67	147
BW07	11/21/2013	8.32	82.91	6.54	631	2.1	3.92	12.45	na	0.43	0.32	-122	95
BW07	12/3/2013	6.53	84.70	6.84	122	1.7	3.27	12.09	14.5	0.20	0.31	-60	157
BW07	12/18/2013	5.63	85.60	6.53	174	1.5	3.03	8.08	13.9	0.11	0.38	-64	153
BW07	1/9/2014	5.85	85.38	7.08	175	1.9	3.62	7.08	12.4	0.25	0.57	-25	192
BW07	2/4/2014	5.33	85.90	6.25	276	1.8	3.55	4.84	11.5	na	0.38	-12	205
BW07	2/17/2014	5.74	85.49	6.70	66.8	1.9	3.76	4.39	10.3	na	0.32	-51	166
BW07	3/5/2014	5.32	85.91	6.70	101	2.1	3.97	7.62	11.2	na	0.10	-85	132
BW07	3/21/2014	5.22	86.01	6.20	130	2.0	3.83	6.52	11.3	na	0.71	8	225
BW07	4/2/2014	5.08	86.15	5.32	113	1.9	3.69	7.05	9.5	na	1.41 R	29	246
BW07	4/18/2014	5.19	86.04	6.64	112	1.7	3.31	10.20	10.2	na	1.33 R	57	274
BW07	4/28/2014	5.72	85.51	6.29	115	1.8	3.38	11.84	11.3	na	1.12 R	19	236
BW07	5/13/2014	6.08	85.15	6.18	97.9	1.5	2.87	18.21	11.6	na	2.00 R	13	223
BW07	6/4/2014	6.00	85.23	6.62	267	2.1	3.95	17.38	na	na	1.90 R	-83	131
BW07	6/18/2014	6.36	84.87	6.50	252	1.8	3.42	21.37	12.2	na	1.53 R	-51	159
BW07	7/2/2014	7.14	84.09	6.55	246	1.8	3.40	22.49	12.5	na	2.76 R	-52	158
BW07	7/18/2014	7.26	83.97	6.33	240	1.9	3.71	18.09	12.9	na	3.39 R	-68	142
BW07	7/29/2014	8.06	83.17	6.38	385	1.8	3.50	18.01	12.9	na	4.60 R	-73	137
BW07	8/13/2014	7.51	83.72	6.38	530	1.9	3.63	19.38	14.1	na	4.60 R	-71	139
BW07	9/8/2014	8.08	83.15	6.07	180	2.0	3.77	17.56	14.0	na	4.91 R	-40	170
BW07	9/24/2014	8.83	82.40	6.53	323	2.3	4.39	17.83	na	na	na	-60	150
BW07	10/7/2014	8.85	82.38	5.52	240	2.0	3.80	16.27	15.5	na	6.68 R	-27	187
BW07	10/21/2014	8.16	83.07	5.58	193	1.9	3.53	16.36	14.9	na	8.40 R	-9	205
BW07	11/3/2014	8.11	83.12	5.80	288	1.9	3.61	14.80	15.0	na	4.23 R	-86	128
BW07	11/20/2014	7.25	83.98	6.04	235	1.8	3.47	12.63	14.6	na	5.86 R	-53	161
BW07	12/2/2014	6.45	84.78	5.92	176	1.5	2.84	13.80	14.7	na	6.72 R	-39	175
BW07	12/18/2014	5.78	85.45	6.67	307	1.7	3.38	9.96	na	na	3.00 R	-44	173
BW07	12/30/2014	6.91	84.32	6.15	146	1.4	3.22	7.93	13.6	na	6.55 R	-17	200
BW07	1/13/2015	5.44	85.79	6.50	147	1.5	2.94	6.58	13.4	na	5.79 R	-12	205
BW07	1/28/2015	5.60	85.63	6.66	213	1.7	3.41	5.55	12.2	na	5.94 R	10	227
BW07	2/10/2015	6.13	85.10	6.08	208	1.7	3.40	5.95	11.8	na	6.13 R	23	240
BW07	3/9/2015	5.04	86.19	4.43	120	1.6	3.18	5.84	10.2	na	6.70 R	-35	182
BW07	3/23/2015	5.49	85.74	5.39	329	1.7	3.28	7.14	na	na	4.03 R	-64	153
BW07	4/8/2015	5.17	86.06	6.48	138	1.4	2.70	7.57	9.8	na	5.89 R	11	228
BW07	4/24/2015	5.45	85.78	7.02	99	1.3	2.54	11.81	11.5	na	3.16 R	-15	202
BW07	5/7/2015	6.25	84.98	6.59	190	1.4	2.65	14.53	11.3	na	0.00	-22	195
BW07	5/20/2015	6.41	84.82	6.00	201	1.3	2.52	14.05	10.9	na	0.01	-10	207
BW07	6/5/2015	5.71	85.52	6.92	211	1.5	2.84	15.68	11.6	na	0.09	-61	156
BW07	6/24/2015	5.55	85.68	6.63	180	1.7	3.26	16.37	na	na	0.00	147	364
BW07	7/7/2015	5.22	86.01	5.07	256	1.3	2.48	24.32	15.6	na	1.20	192	409
BW07	7/23/2015	7.23	84.00	6.77	266	1.7	3.23	21.39	15.4	na	0.44	6	223
BW07	8/19/2015	8.21	83.02	4.45	338	3.7	6.78	22.15	13.7	na	1.86 R	-37	180
BW07	9/9/2015	8.59	82.64	7.37	386	1.6	3.10	22.78	14.6	na	3.26 R	-91	126
BW07	9/28/2015	9.42	81.81	6.17	343	1.8	3.43	20.55	na	na	1.83 R	-93	124
BW07	10/13/2015	7.32	83.91	6.48	385	1.8	3.32	18.23	16.1	na	0.25	-88	129
BW07	11/2/2015	7.24	83.99	5.28	429	1.7	3.31	15.36	15.7	na	0.00	-40	177
BW07	11/17/2015	6.56	84.67	5.39	398	1.4	2.68	13.89	14.7	na	0.49	-58	159
BW07	12/4/2015	5.75	85.48	5.94	594	1.5	2.83	12.24	14.1	na	0.77	-57	160
BW07	12/21/2015	7.19	84.04	6.02	628	1.0	2.74	12.93	14.6	na	0.81	-46	171
BW07	1/15/2016	6.05	85.18	6.34	698	2.1	4.08	10.27	na	na	0.00	-65	152
BW07	2/5/2016	4.71	86.52	6.69	>999	1.4	3.74	6.37	10.5	na	0.35	-43	174
BW07	2/18/2016	5.57	85.66	6.23	398	1.8	3.61	6.28	11.5	na	0.41	-13	204
BW07	3/7/2016	5.85	85.38	5.21	181	0.5	0.935	10.69	11.4	na	0.25	-27	190
BW07	3/22/2016	5.92	85.31	4.64	387	0.5	1.06	9.95	10.0	na	0.33	-4	213
BW07	4/8/2016	5.46	85.77	6.90	605	1.7	3.32	9.93	na	na	0.01	-88	129
BW07	5/24/2016	4.95	86.28	6.88	88.9	1.4	3.10	16.89	12.7	na	0.04	-76	141
BW07	6/8/2016	4.97	86.26	6.83	232	1.4	3.19	16.23	13.1	na	0.42	-52	165
BW07	6/24/2016	8.15	83.08	6.99	593	1.8	3.47	19.74	na	na	0.01	-67	150
BW07	7/13/2016	7.20	84.03	6.77	362	1.4	2.65	20.67	15.6	na	0.53	4	221
BW07	7/27/2016	7.75	83.48	6.28	813	1.5	2.95	17.53	13.9	na	0.01	-14	203
BW07	8/18/2016	7.03	84.20	6.45	877	1.6	3.07	20.44	15.0	na	0.97	-86	131
BW07	8/31/2016	7.52	83.71	5.66	>1000	1.7	3.25	19.67	15.8	na	1.51	-95	122
BW07	9/22/2016	9.51	81.72	5.68	736	1.9	3.62	19.72	na	na	1.20	-36	181

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW08	10/22/2013	7.55	84.29	6.06	>999	2.1	3.97	15.63	na	na	0.99	-72	142
BW08	11/14/2013	8.01	83.83	6.05	205	2.4	4.47	12.80	16.2	0.29	0.19	-84	130
BW08	11/21/2013	8.24	83.60	6.52	442	2.3	4.31	12.93	na	0.49	0.35	-115	99
BW08	12/3/2013	6.99	84.85	6.85	151	2.1	4.02	12.19	14.6	0.06	0.37	-66	151
BW08	12/18/2013	5.73	86.11	6.45	228	2.4	4.54	7.80	13.6	0.03	0.31	-67	150
BW08	1/9/2014	5.74	86.10	7.10	103	2.2	4.10	7.24	12.5	0.27	0.57	-34	183
BW08	2/4/2014	5.25	86.59	6.36	370	1.8	3.55	5.47	12.1	na	0.36	-41	176
BW08	2/17/2014	5.50	86.34	6.73	83	1.6	3.22	4.82	11.3	na	0.58	-51	166
BW08	3/5/2014	5.16	86.68	6.46	252	2.0	3.87	6.70	9.3	na	0.07	-80	137
BW08	3/21/2014	5.06	86.78	6.25	80.2	1.6	3.15	6.55	12.1	na	0.72	-1	216
BW08	4/2/2014	4.97	86.87	5.22	96.2	1.5	2.95	7.13	10.8	na	1.16 R	37	254
BW08	4/18/2014	5.03	86.81	6.53	82.4	1.4	2.66	10.37	10.5	na	1.45 R	74	291
BW08	4/28/2014	5.39	86.45	6.19	109	1.4	2.65	12.76	11.0	na	1.40 R	37	251
BW08	5/13/2014	5.60	86.24	6.14	102	1.1	2.18	20.09	11.0	na	1.90 R	9	219
BW08	6/4/2014	5.56	86.28	6.73	215	2.3	4.24	19.91	na	na	1.06 R	-85	125
BW08	6/18/2014	5.81	86.03	6.45	167	1.4	2.79	22.52	12.4	na	1.42 R	-45	162
BW08	7/2/2014	6.49	85.35	6.57	294	1.4	2.70	23.60	12.7	na	2.38 R	-47	160
BW08	7/18/2014	6.72	85.12	6.23	267	1.7	3.26	18.62	12.6	na	4.02 R	-48	162
BW08	7/29/2014	7.31	84.53	6.22	281	1.3	2.44	19.56	13.2	na	4.86 R	-52	158
BW08	8/13/2014	6.13	85.71	6.20	215	1.5	2.83	23.47	13.4	na	4.54 R	-56	151
BW08	9/8/2014	7.64	84.20	6.18	459	1.5	2.92	17.62	14.5	na	4.74 R	-44	166
BW08	9/24/2014	8.23	83.61	6.50	0	2.0	3.87	18.85	na	na	1.47 R	-54	156
BW08	10/7/2014	8.33	83.51	5.42	224	1.6	2.99	16.76	15.2	na	6.92 R	-16	198
BW08	10/21/2014	7.90	83.94	5.49	272	1.4	2.68	17.03	15.0	na	8.62 R	1	215
BW08	11/3/2014	8.19	83.65	5.58	296	1.6	3.00	14.56	15.0	na	4.31 R	-59	155
BW08	11/20/2014	7.39	84.45	5.88	289	1.5	2.82	12.68	14.9	na	5.59 R	-38	176
BW08	12/2/2014	6.96	84.88	5.82	239	1.4	2.71	14.34	14.9	na	6.87 R	-31	183
BW08	12/18/2014	5.75	86.09	6.45	353	1.0	1.96	10.56	na	na	2.90 R	-25	192
BW08	12/30/2014	5.76	86.08	6.00	144	1.2	2.34	7.56	13.9	na	6.99 R	2	219
BW08	1/13/2015	5.35	86.49	6.72	146	0.8	1.57	6.17	13.8	na	5.60 R	-14	203
BW08	1/28/2015	5.48	86.36	6.55	146	0.9	1.86	4.74	13.2	na	6.26 R	34	251
BW08	2/10/2015	5.78	86.06	6.04	109	0.7	1.44	5.01	12.2	na	6.57 R	51	268
BW08	3/9/2015	5.10	86.74	4.29	95	0.7	1.42	5.87	10.0	na	7.21 R	-2	215
BW08	3/23/2015	5.35	86.49	5.25	227	1.0	1.94	6.85	na	na	3.54 R	-50	167
BW08	4/8/2015	4.99	86.85	6.64	98.2	0.5	0.971	7.97	9.7	na	6.21 R	24	241
BW08	4/24/2015	5.25	86.59	6.93	78.6	0.5	0.974	12.19	10.7	na	3.52 R	11	228
BW08	5/7/2015	5.81	86.03	6.63	112	0.4	0.819	16.20	11.0	na	0.12	5	222
BW08	5/20/2015	5.89	85.95	6.13	131	0.4	0.812	16.13	11.4	na	0.07	14	231
BW08	6/5/2015	5.38	86.46	7.29	116	0.4	0.902	16.93	12.0	na	0.06	-68	149
BW08	6/23/2015	4.75	87.09	6.46	150	0.5	0.988	21.89	na	na	0.02	161	378
BW08	7/7/2015	4.85	86.99	4.49	172	1.1	2.110	22.64	13.6	na	1.16	221	438
BW08	7/23/2015	6.56	85.28	6.70	139	0.3	0.703	21.67	15.2	na	0.86	59	276
BW08	8/19/2015	7.61	84.23	4.31	199	1.3	2.480	22.67	15.6	na	1.97 R	10	227
BW08	9/9/2015	8.17	83.67	7.38	318	0.8	1.680	22.25	15.1	na	1.68 R	-71	146
BW08	9/28/2015	8.51	83.33	6.02	643	1.2	2.390	20.61	na	na	1.83 R	-69	148
BW08	10/13/2015	6.68	85.16	6.09	752	0.7	2.310	17.31	15.9	na	0.18	-48	169
BW08	11/2/2015	7.20	84.64	5.45	413	1.0	2.030	15.56	16.0	na	0.00	-34	183
BW08	11/17/2015	6.59	85.25	5.58	352	0.7	1.490	14.17	15.6	na	0.51	-42	175
BW08	12/4/2015	5.71	86.13	6.25	345	0.5	0.931	11.48	14.6	na	0.65	-39	178
BW08	12/21/2015	7.17	84.67	6.36	371	0.5	0.899	12.64	14.1	na	0.69	-47	170
BW08	1/15/2016	5.77	86.07	6.26	514	0.8	1.020	9.89	na	na	0.07	-28	189
BW08	2/5/2016	4.86	86.98	6.79	>999	0.4	0.992	6.13	11.3	na	0.51	-44	173
BW08	2/18/2016	5.58	86.26	5.58	365	0.4	0.917	5.47	11.1	na	0.17	1	218
BW08	3/7/2016	5.72	86.12	5.36	85	0.3	0.627	10.75	11.0	na	0.26	4	221
BW08	3/22/2016	5.71	86.13	4.80	254	0.3	0.685	9.56	9.7	na	0.29	9	226
BW08	4/8/2016	5.70	86.14	6.85	445	0.5	1.11	9.85	na	na	0.29	-51	166
BW08	5/24/2016	5.23	86.61	7.21	355	0.5	0.961	17.32	12.2	na	0.01	-56	161
BW08	6/8/2016	6.25	85.59	6.95	356	0.3	0.708	17.01	12.2	na	0.36	-1	216
BW08	6/23/2016	6.55	85.29	6.81	633	0.7	1.42	16.13	na	na	0.01	-44	173
BW08	7/13/2016	6.84	85.00	6.75	172	0.3	0.71	22.01	15.5	na	0.86	59	276
BW08	7/27/2016	7.30	84.54	6.35	796	0.8	1.53	18.88	15.5	na	0.01	10	227
BW08	8/18/2016	7.03	84.81	6.18	829	0.8	1.67	20.79	17.5	na	1.20	-49	168
BW08	8/31/2016	7.37	84.47	5.49	901	1.1	2.29	21.20	16.2	na	1.81	-67	150
BW08	9/22/2016	8.86	82.98	5.40	986	1.3	2.59	18.77	na	na	1.20	-2	215

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW09	10/22/2013	7.96	84.41	5.38	>999	0.6	1.21	16.65	na	na	0.60	-18	196
BW09	11/14/2013	8.38	83.99	5.49	233	0.4	0.77	13.01	16.1	0.27	0.50	30	244
BW09	11/21/2013	8.42	83.95	5.85	205	0.5	1.05	12.85	na	0.97	0.43	-11	203
BW09	12/3/2013	9.53	82.84	6.10	488	0.6	1.16	12.48	14.6	0.13	0.50	20	237
BW09	12/18/2013	6.23	86.14	6.57	189	0.6	1.27	6.90	12.7	0.11	0.40	-47	170
BW09	1/9/2014	6.01	86.36	6.89	20	0.5	0.970	6.10	11.3	0.30	0.60	-9	208
BW09	2/4/2014	5.38	86.99	6.15	253	0.5	1.06	6.32	8.4	0.34	3.58	21	238
BW09	2/17/2014	5.54	86.83	6.11	201	0.4	0.910	5.85	8.5	0.41	4.45	34	251
BW09	3/5/2014	5.25	87.12	6.36	12.4	0.4	0.812	6.37	7.6	na	0.04	-26	191
BW09	3/21/2014	5.11	87.26	5.94	>999	0.5	0.992	7.79	9.3	0.52	2.51	35	252
BW09	4/2/2014	5.08	87.29	5.05	95.7	0.4	0.920	7.53	8.4	na	4.88 R	80	297
BW09	4/18/2014	5.11	87.26	6.42	>999	0.5	0.943	9.82	9.7	1.08	5.68 R	111	328
BW09	4/28/2014	5.42	86.95	6.06	84.6	0.5	0.957	12.46	10.3	1.50	4.36 R	58	275
BW09	5/13/2014	5.71	86.66	5.87	142	0.4	0.873	19.72	12.2	1.65	4.70 R	43	253
BW09	6/4/2014	5.85	86.52	6.21	46.5	0.4	0.907	17.41	na	na	1.04 R	-25	189
BW09	6/18/2014	6.01	86.36	6.38	>999	0.6	1.17	20.13	13.1	1.39	4.22 R	-21	189
BW09	7/2/2014	6.67	85.70	6.31	0	0.7	1.32	22.74	12.9	2.95	4.06 R	-21	186
BW09	7/18/2014	7.02	85.35	5.92	126	0.5	0.98	22.29	13.4	3.70	3.99 R	-15	195
BW09	7/29/2014	7.79	84.58	6.01	188	0.6	1.12	22.64	13.8	na	4.74 R	-36	171
BW09	8/13/2014	7.11	85.26	6.42	143	0.5	0.982	25.29	14.1	3.37	4.33 R	-20	187
BW09	9/8/2014	7.93	84.44	5.94	101	0.6	1.12	19.08	14.9	5.82	4.66 R	-23	187
BW09	9/24/2014	8.52	83.85	5.97	538	0.7	1.47	17.22	na	na	1.75 R	-11	203
BW09	10/7/2014	8.65	83.72	5.48	77.8	0.6	1.17	17.70	16.1	5.99	7.01 R	-14	196
BW09	10/21/2014	8.22	84.15	5.30	98	0.5	0.974	17.65	16.1	7.13	9.05 R	30	240
BW09	11/3/2014	8.19	84.18	5.49	98.3	0.4	0.859	14.81	15.5	7.30	5.51 R	-10	204
BW09	11/20/2014	7.69	84.68	5.45	133	0.3	0.538	11.63	15.3	8.59	6.36 R	35	249
BW09	12/2/2014	7.12	85.25	5.59	108	0.4	0.761	14.46	15.2	8.03	7.44 R	11	225
BW09	12/18/2014	6.11	86.26	6.08	188	0.6	1.18	10.38	na	na	2.97 R	3	220
BW09	12/30/2014	5.98	86.39	5.92	130	0.4	0.909	7.68	13.5	6.26	8.01 R	21	238
BW09	1/13/2015	5.44	86.93	6.78	69.1	0.3	0.652	4.95	12.8	5.40	5.43 R	-14	203
BW09	1/28/2015	5.41	86.96	6.61	85.2	0.3	0.662	2.82	11.3	3.81	7.32 R	50	264
BW09	2/10/2015	5.73	86.64	6.12	72.2	0.3	0.618	2.91	11.3	5.47	7.60 R	66	280
BW09	3/9/2015	4.95	87.42	4.26	54.9	0.3	0.573	4.36	9.6	6.23	8.00 R	12	229
BW09	3/23/2015	5.19	87.18	4.96	207	0.3	0.639	5.82	na	na	5.50 R	-13	204
BW09	4/8/2015	4.86	87.51	6.76	95.4	0.3	0.539	8.61	9.9	3.65	7.24 R	30	247
BW09	4/24/2015	5.29	87.08	7.02	95.3	0.2	0.493	13.55	10.4	3.70	4.10 R	15	232
BW09	5/7/2015	5.96	86.41	6.80	104	0.2	0.483	17.49	11.1	0.27	1.89	8	225
BW09	5/20/2015	6.20	86.17	6.24	107	0.2	0.484	16.97	11.2	0.34	1.15	19	236
BW09	6/5/2015	5.72	86.65	6.94	97.7	0.3	0.670	17.68	12.6	0.41	0.49	-44	173
BW09	6/23/2015	5.65	86.72	6.16	256	0.3	0.701	22.45	na	na	0.14	188	405
BW09	7/7/2015	5.35	87.02	4.60	269	0.9	1.800	23.94	15.7	1.39	0.94	215	432
BW09	7/23/2015	6.88	85.49	6.80	124	0.3	0.682	24.07	17.5	1.41	0.91	66	283
BW09	8/19/2015	8.11	84.26	4.32	160	1.2	2.320	23.68	15.8	1.25	2.38 R	11	228
BW09	9/9/2015	8.66	83.71	7.26	185	0.6	1.170	24.73	15.9	2.94	4.23 R	-59	158
BW09	9/28/2015	8.90	83.47	5.84	396	0.5	1.020	20.22	na	na	1.76 R	-34	183
BW09	10/13/2015	6.94	85.43	5.81	398	0.8	1.340	17.92	16.4	0.09	0.19	-38	179
BW09	11/2/2015	7.56	84.81	5.59	283	0.7	1.490	15.99	16.5	0.68	0.00	-22	195
BW09	11/17/2015	6.88	85.49	5.86	304	0.4	0.847	14.24	15.8	0.40	0.53	-37	180
BW09	12/4/2015	5.97	86.40	6.52	340	0.4	0.753	11.34	14.6	0.78	0.86	-59	158
BW09	12/21/2015	7.53	84.84	6.49	371	0.5	0.744	12.33	14.4	0.76	0.81	-62	155
BW09	1/15/2016	6.01	86.36	6.39	500	0.5	1.080	9.14	na	na	0.19	-31	186
BW09	2/5/2016	4.91	87.46	6.09	>999	0.4	0.898	5.84	9.5	0.36	0.58	41	258
BW09	2/18/2016	5.62	86.75	6.23	370	0.4	0.743	4.50	11.8	0.41	0.84	-7	210
BW09	3/7/2016	5.85	86.52	5.76	50	0.3	0.543	10.52	9.3	0.67	0.43	-13	204
BW09	3/22/2016	5.86	86.51	5.23	254	0.2	0.511	9.51	9.6	0.54	0..38	-6	211
BW09	4/8/2016	5.70	86.67	7.13	495	0.4	0.770	9.90	na	na	0.08	-66	151
BW09	5/24/2016	5.69	86.68	7.71	381	0.4	0.797	18.78	13.0	0.03	0.48	-75	142
BW09	6/8/2016	5.71	86.66	6.25	389	0.4	0.559	18.19	12.7	0.41	0.51	-37	180
BW09	6/23/2016	7.09	85.28	6.69	574	0.4	0.837	18.78	na	na	0.01	-36	181
BW09	7/13/2016	7.16	85.21	6.72	137	0.3	0.68	23.15	15.1	0.91	0.83	77	294
BW09	7/27/2016	7.79	84.58	6.79	760	0.5	1.10	21.72	17.1	0.90	1.05	22	239
BW09	8/18/2016	6.86	85.51	6.14	800	0.6	1.21	21.46	18.0	1.20	1.67	-31	186
BW09	8/31/2016	7.81	84.56	5.50	883	0.6	1.24	23.94	19.3	1.22	1.04	-37	180
BW09	9/22/2016	10.07	82.30	5.27	712	0.7	1.44	20.67	na	na	1.35	25	242

Site	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T, U52 (◦C)	T, Downhole (◦C)	DO: Pre-purge (mg/L)	DO: Post-purge (mg/L)	O/R Potential (mV)	O/R Potential, Eh (mV)
BW10	10/22/2013	7.96	85.27	5.62	>999	1.1	2.08	17.05	na	na	0.93	-21	193
BW10	11/14/2013	8.95	84.28	5.75	511	1.3	2.59	13.19	15.8	0.26	0.19	-36	178
BW10	11/21/2013	9.20	84.03	6.31	291	1.5	2.95	12.10	na	1.16	0.87	-74	143
BW10	12/3/2013	7.55	85.68	6.78	242	1.1	2.24	13.19	14.6	0.29	0.34	-57	157
BW10	12/18/2013	5.82	87.41	6.35	110	1.6	3.16	7.08	13.8	0.11	0.26	-38	179
BW10	1/9/2014	5.65	87.58	6.87	93	1.1	2.20	5.84	12.6	0.29	0.56	-13	204
BW10	2/4/2014	5.15	88.08	6.34	134	0.8	1.73	4.22	11.2	na	0.33	-5	212
BW10	2/17/2014	5.21	88.02	6.82	34.6	0.7	1.44	3.56	10.4	na	0.48	-41	176
BW10	3/5/2014	4.82	88.41	6.70	96.8	1.3	2.54	5.00	7.7	na	0.10	-46	171
BW10	3/21/2014	4.74	88.49	6.37	67.8	0.9	1.90	6.36	10.7	na	0.82	-2	215
BW10	4/2/2014	4.94	88.29	4.98	48.7	0.5	0.954	7.26	10.7	na	1.62 R	67	284
BW10	4/18/2014	5.24	87.99	6.18	95.9	0.3	0.621	10.77	10.8	na	1.90 R	93	310
BW10	4/28/2014	5.66	87.57	5.84	54.8	0.3	0.610	13.69	11.0	na	2.26 R	78	292
BW10	5/13/2014	5.95	87.28	5.75	48.6	0.3	0.643	20.95	11.7	na	2.05 R	41	251
BW10	6/4/2014	5.83	87.40	6.56	360	0.8	1.66	18.61	na	na	1.07 R	-46	164
BW10	6/18/2014	6.15	87.08	6.41	130	0.6	1.20	23.31	12.9	na	1.50 R	-28	179
BW10	7/2/2014	6.85	86.38	6.49	215	0.9	1.74	25.24	13.1	na	2.18 R	-38	169
BW10	7/18/2014	7.12	86.11	6.24	142	1.1	2.14	22.02	13.3	na	3.66 R	-46	164
BW10	7/29/2014	7.98	85.25	6.31	175	1.1	2.12	21.06	14.0	na	5.73 R	-59	151
BW10	8/13/2014	7.50	85.73	6.17	201	1.1	2.13	24.87	14.5	na	4.55 R	-39	168
BW10	9/8/2014	8.39	84.84	5.90	128	1.0	1.95	18.74	15.2	na	4.74 R	-24	186
BW10	9/24/2014	9.26	83.97	6.26	423	1.3	2.52	18.14	na	na	1.62 R	-33	177
BW10	10/7/2014	9.32	83.91	6.18	88.2	0.8	1.64	18.03	16.2	na	7.39 R	-47	163
BW10	10/21/2014	8.58	84.65	5.18	111	0.6	1.21	19.15	16.0	na	9.49 R	55	265
BW10	11/3/2014	8.73	84.50	6.31	108	0.6	1.24	15.17	15.6	na	6.13 R	-50	164
BW10	11/20/2014	8.12	85.11	5.61	163	0.6	1.22	12.06	15.7	na	7.87 R	11	228
BW10	12/2/2014	7.52	85.71	5.66	109	0.6	1.19	16.39	15.4	na	8.17 R	19	233
BW10	12/18/2014	6.45	86.78	6.45	179	1.1	2.23	11.59	na	na	3.29 R	-8	209
BW10	12/30/2014	6.14	87.09	6.04	102	0.7	1.38	6.41	13.6	na	9.74 R	60	277
BW10	1/13/2015	5.58	87.65	6.23	75.7	0.3	0.626	4.35	13.1	na	5.34 R	20	237
BW10	1/28/2015	5.49	87.74	6.32	91	0.3	0.731	1.75	11.1	na	9.43 R	91	308
BW10	2/10/2015	6.01	87.22	5.88	75.1	0.5	0.991	3.04	11.7	na	8.90 R	93	310
BW10	3/9/2015	4.95	88.28	4.14	68.6	0.3	0.558	4.03	10.7	na	8.82 R	51	268
BW10	3/23/2015	5.55	87.68	5.12	75.1	0.5	1.02	6.15	na	na	3.07 R	-24	193
BW10	4/8/2015	5.25	87.98	6.66	75	0.3	0.597	9.15	10.6	na	8.35 R	67	284
BW10	4/24/2015	5.46	87.77	6.71	75.7	0.3	0.574	14.05	11.2	na	4.86 R	51	268
BW10	5/7/2015	6.39	86.84	6.75	91.4	0.3	0.665	19.23	11.6	na	0.85	45	262
BW10	5/20/2015	6.78	86.45	6.31	119	0.3	0.629	18.24	11.8	na	0.60	43	260
BW10	6/5/2015	6.13	87.10	6.67	131	0.5	1.090	18.04	13.4	na	0.16	-33	184
BW10	6/23/2015	4.78	88.45	6.39	82	0.5	1.030	20.54	na	na	0.00	153	370
BW10	7/7/2015	5.35	87.88	4.61	>999	1.3	2.470	24.51	16.3	na	0.91	240	457
BW10	7/23/2015	7.27	85.96	7.16	186	0.5	1.090	23.28	15.7	na	1.71 R	88	305
BW10	8/19/2015	8.66	84.57	4.53	163	1.5	2.950	24.63	16.2	na	2.26 R	0	217
BW10	9/9/2015	9.37	83.86	7.20	204	0.7	1.460	26.50	20.3	na	4.36 R	-51	166
BW10	9/28/2015	9.92	83.31	5.97	408	1.0	1.950	21.04	na	na	1.67 R	-57	160
BW10	10/13/2015	7.25	85.98	6.12	322	0.7	1.990	18.20	16.2	na	0.54	-62	155
BW10	11/2/2015	8.19	85.04	5.55	392	1.0	1.940	16.46	16.6	na	0.00	-5	212
BW10	11/17/2015	7.26	85.97	6.09	320	0.9	1.740	14.64	15.6	na	0.29	-62	155
BW10	12/4/2015	5.89	87.34	na	na	na	na	na	na	na	na	na	na
BW10	12/21/2015	8.15	85.08	6.13	367	0.5	1.730	14.48	14.1	na	0.64	-65	152
BW10	1/15/2016	4.68	88.55	6.42	515	1.2	2.290	9.04	na	na	0.01	-56	161
BW10	2/5/2016	4.40	88.83	6.82	>999	0.7	1.43	6.38	10.8	na	0.48	-42	175
BW10	2/18/2016	5.41	87.82	6.32	387	0.8	1.73	4.83	11.2	na	0.33	-5	212
BW10	3/7/2016	5.81	87.42	5.81	>999	0.3	0.639	11.27	9.3	na	0.33	7	224
BW10	3/22/2016	6.04	87.19	5.30	246	0.2	0.496	9.58	9.4	na	0.46	18	235
BW10	4/8/2016	5.90	87.33	7.18	481	1.2	2.46	9.32	na	na	0.01	-78	139
BW10	5/24/2016	5.58	87.65	7.91	328	0.5	0.958	18.63	12.7	na	0.70	-76	141
BW10	6/8/2016	5.65	87.58	6.85	316	0.5	0.899	18.27	12.1	na	0.56	-14	203
BW10	6/24/2016	7.82	85.41	6.88	600	0.6	1.23	19.68	na	na	0.01	-52	165
BW10	9/22/2016	6.75	86.48	4.28	610	0.3	0.708	17.22	na	na	1.18	160	377

Appendix A10: Transect well (TW) field measurements, physical parameters

Well	Date	DTGW (fbgs)	GW Elevation	pH	Turbidity (NTU)	Salinity (ppt)	Sp. Cond (mS/cm)	T (◦C)	DO (mg/L)	O/R Potential (mV)
TW6	11/20/2013	6.83	83.58	5.27	>999	0.3	0.657	12.55	0.66	83
	2/26/2014	4.38	86.03	5.02	691	0.3	6.89	7.60	0.08	107
	6/4/2014	4.74	85.67	4.97	977	0.3	0.620	14.18	0.62	105
	9/24/2014	7.80	82.61	4.95	0	0.3	0.671	16.05	1.25 R	117
	12/18/2014	4.90	85.51	4.34	0	0.3	0.621	11.44	3.58 R	142
	3/23/2015	4.49	85.92	3.02	0	0.3	0.718	7.76	1.36 R	125
	6/23/2015	4.61	85.80	5.32	>999	0.3	0.571	21.76	0.14	99
	9/30/2015	7.37	83.04	4.33	>999	0.3	0.624	17.72	0.01	123
	1/14/2016	5.14	85.27	3.63	>999	0.3	0.702	11.24	0.01	91
	4/6/2016	5.27	85.14	3.76	>999	0.3	0.620	10.35	0.01	51
	6/30/2016	5.86	84.55	5.18	>999	0.3	0.678	17.96	0.43	130
	9/21/2016	8.56	81.85	4.11	>1000	0.3	0.648	17.48	1.34	153
TW5	11/20/2013	7.13	83.58	5.10	0	0.3	0.660	12.75	0.77	111
	2/26/2014	4.62	86.09	5.08	0	0.3	0.674	5.61	0.14	106
	6/4/2014	5.02	85.69	5.01	0	0.3	0.613	16.81	0.71	73
	9/24/2014	8.06	82.65	5.00	0	0.3	0.677	15.58	1.70 R	103
	12/18/2014	5.20	85.51	4.38	>999	0.3	0.611	11.16	2.20 R	144
	3/23/2015	4.80	85.91	3.00	>999	0.3	0.714	8.02	1.17 R	128
	6/23/2015	4.80	85.91	5.22	322	0.3	0.592	17.80	0.01	114
	9/30/2015	7.69	83.02	4.48	>999	0.3	0.636	18.28	0.01	87
	1/14/2016	5.39	85.32	3.94	>999	0.3	0.700	11.54	0.01	65
	4/6/2016	5.51	85.20	3.96	>999	0.3	0.619	11.26	0.01	21
	6/29/2016	6.20	84.51	5.16	>999	0.3	0.696	14.10	0.09	125
	9/21/2016	8.80	81.91	4.38	>1000	0.3	0.669	17.65	1.15	131
TW4	11/20/2013	6.82	83.56	6.31	0	1.5	2.92	16.08	0.23	-144
	2/26/2014	5.72	84.66	6.13	0	0.7	1.41	7.63	0.23	-51
	6/4/2014	4.75	85.63	5.78	>999	0.6	1.05	14.83	0.83	-28
	9/24/2014	7.80	82.58	5.82	0	0.5	1.00	20.13	1.24 R	-26
	12/18/2014	4.92	85.46	5.76	>999	0.8	1.59	11.44	2.85 R	-8
	3/23/2015	4.52	85.86	3.94	557	0.6	1.18	9.11	0.83 R	14
	6/22/2015	5.05	85.33	4.93	222	0.3	0.659	20.83	0.01	6
	9/30/2015	7.12	83.26	5.10	775	0.6	1.28	18.04	0.01	13
	1/14/2016	6.14	84.24	4.88	>999	0.5	1.02	11.76	0.01	-21
	4/6/2016	5.97	84.41	5.04	>999	0.4	0.759	13.80	0.01	-50
	6/29/2016	5.88	84.50	6.00	>999	0.4	0.906	14.61	0.31	34
	9/21/2016	7.91	82.47	5.43	>1000	0.5	0.934	16.05	1.40	42
TW3	11/20/2013	7.18	83.32	6.57	>999	2.1	3.91	15.96	0.86	-187
	2/26/2014	4.71	85.79	6.54	0	1.9	3.65	6.97	0.26	-98
	6/4/2014	5.13	85.37	6.37	0	1.6	3.08	16.16	0.82	-95
	9/24/2014	8.25	82.25	6.54	>999	1.9	3.52	19.56	1.20 R	-86
	12/18/2014	5.18	85.32	6.58	684	1.8	3.47	11.45	3.28 R	-40
	3/23/2015	4.80	85.70	4.82	>999	1.5	2.98	10.05	1.05 R	-73
	6/22/2015	5.90	84.60	5.80	592	1.2	2.39	20.19	0.00	-92
	9/29/2015	8.56	81.94	5.37	>999	1.5	2.87	18.38	0.00	-71
	1/14/2016	5.29	85.21	5.03	891	1.3	2.58	10.60	0.01	-63
	4/6/2016	5.51	84.99	5.26	848	1.1	2.11	13.73	0.01	-95
	6/30/2016	6.06	84.44	6.14	>999	1	1.96	13.17	0.55	-31
	9/21/2016	8.5	82.00	5.65	>1000	1	2	17.92	1.38	-10
TW2	11/20/2013	7.22	83.47	5.93	0	0.7	1.45	15.86	0.48	-67
	2/26/2014	5.34	85.35	6.29	0	1.2	2.34	6.94	0.22	-54
	6/4/2014	5.60	85.09	6.12	>999	0.8	1.61	19.29	0.84	-56
	9/24/2014	8.25	82.44	6.29	796	1.1	2.14	18.19	1.13 R	-68
	12/18/2014	5.57	85.12	6.42	661	1.0	1.98	12.11	2.80 R	-17
	3/23/2015	5.22	85.47	5.13	486	0.8	1.64	6.56	2.34 R	-23
	6/22/2015	5.51	85.18	5.45	703	0.6	1.14	22.73	0.21	-46
	9/29/2015	9.20	81.49	5.28	896	0.7	1.38	17.39	0.21	-37
	1/14/2016	5.80	84.89	4.95	>999	0.6	1.27	11.07	0.01	-32
	4/6/2016	6.05	84.64	5.1	952	0.5	1.02	10.94	0.01	-45
	6/30/2016	6.49	84.20	6.15	926	0.6	1.26	12.92	0.31	-32
	9/21/2016	8.51	82.18	5.49	>1000	0.7	1.49	16.61	1.75	18
TW1	11/20/2013	7.28	83.46	5.91	>999	0.7	1.32	16.41	0.55	-79
	2/26/2014	5.00	85.74	6.25	0	1.2	2.37	6.78	0.23	-48
	6/4/2014	5.58	85.16	6.06	0	0.9	1.76	16.19	0.76	-47
	9/24/2014	8.30	82.44	6.22	381	1.1	2.08	16.97	1.51 R	-55
	12/18/2014	5.62	85.12	6.32	962	1	1.99	12.2	2.79 R	-16
	3/23/2015	5.83	84.91	5.11	>999	0.8	1.63	6.24	1.94 R	-28
	6/22/2015	5.22	85.52	5.23	>999	0.5	1.08	15.59	0.01	-21
	9/29/2015	8.55	81.86	5.35	>999	0.7	1.35	17.39	0.01	-39
	1/14/2016	5.50	84.91	5.16	>999	0.7	1.36	10.72	0.01	-32
	4/6/2016	5.91	84.5	5.19	>999	0.5	1.02	9.88	0.01	-36
	6/30/2016	6.56	84.18	6.07	>999	0.6	1.27	13.31	0.29	-28
	9/20/2016	8.60	82.14	6.9	>1000	0.7	1.4	18.02	1.30	13
TW00	9/20/2016	7.99	90.45	6.77	>1000	0.7	1.34	17.96	1.19	21

Appendix A11: Remedial investigation well (MW) field measurments, physical parameters

Well	Date	DO (mg/L)	T (°C)
MW2	2/26/2014	2.10	10.1
	6/4/2014	2.1	10.1
	9/24/2014	4.38	14.0
	12/18/2014	5.93	13.7
	3/23/2015	9.19	10.8
	6/19/2015	0.56	12.9
	9/25/2015	2.86	14.2
	1/20/2016	3.94	12.7
	4/6/2016	3.91	11.4
	6/23/2016	3.91	16.2
	9/20/2016	4.79	17.3
MW3	2/26/2014	9.23	
	6/4/2014	8.46	11.6
	9/24/2014	7.73	13.3
	12/18/2014	9.43	13.3
	3/23/2015	10.59	10.6
	6/19/2015	7.71	12.1
	9/25/2015	7.02	15.9
	1/20/2016	8.06	11.4
	4/6/2016	9.06	11.1
	6/23/2016	8.12	13.3
	9/20/2016	7.8	16.9
MW4	2/26/2014	5.50	
	6/4/2014	3.7	11.8
	9/24/2014	3.67	13.8
	12/18/2014	9.29	13.7
	3/23/2015	10.69	9.1
	6/19/2015	3.23	13.5
	9/25/2015	4.02	16.5
	1/20/2016	6.37	11.6
	4/6/2016	6.07	10.8
	6/23/2016	3.13	14.0
	9/20/2016	4.27	20.4
MW5	2/26/2014	7.07	12.6
	6/4/2014	4.07	13.2
	9/24/2014	8.87	13.4
	12/18/2014	9.75	13.0
	3/23/2015	10.71	12.8
	6/19/2015	8.87	13.3
	9/25/2015	3.25	15.3
	1/20/2016	5.26	12.4
	4/6/2016	5.02	12.9
	6/23/2016	6.11	16.8
	9/20/2016	7.8	13.3

Well	Date	DO (mg/L)	T (°C)
MW6	2/26/2014	1.36	10.7
	6/4/2014	2.80	11.8
	9/24/2014	4.88	14.9
	12/18/2014	5.89	13.6
	3/23/2015	9.13	10.0
	6/19/2015	0.50	12.3
	9/25/2015	3.25	15.3
	1/20/2016	2.01	10.6
	4/6/2016	0.34	11.3
	6/23/2016	4.36	17.2
	9/20/2016	4.56	18.5
MW7	2/26/2014	3.57	11.3
	6/4/2014	2.69	11.7
	9/24/2014	5.97	13.2
	12/18/2014	5.95	13.3
	3/23/2015	5.97	11.5
	6/19/2015	0.45	12.1
	9/25/2015	3.91	13.6
	1/20/2016	3.87	11.3
	4/6/2016	2.07	10.8
	6/23/2016	4.66	20.3
	9/20/2016	3.48	16.2
MW8	2/26/2014	3.70	10.6
	6/4/2014	3.05	11.6
	9/24/2014	7.43	14.0
	12/18/2014	7.02	12.9
	3/23/2015	9.32	11.1
	6/19/2015	0.53	11.9
	9/25/2015	3.43	15.4
	1/20/2016	3.60	11.4
	4/6/2016	2.74	10.6
	6/23/2016	3.61	18.0
	9/20/2016	4.35	17.4
MW9	2/26/2014	0.65	11.0
	6/4/2014	2.73	11.0
	9/24/2014	4.99	14.2
	12/18/2014	6.88	13.2
	3/23/2015	9.26	10.6
	6/19/2015	0.60	12.0
	9/25/2015	3.09	14.6
	1/20/2016	0.56	12.6
	4/6/2016	0.14	10.4
	6/23/2016	4.99	19.4
	9/20/2016	4.54	17.2

Appendix A12: Biowall well (BW) field measurements, geochemical parameters

Site	Date	Total Alkalinity (mg/L CaCO3)	Total Organic Carbon (TOC) (mg/L)	Nitrate (mg/L)*	Nitrite (mg/L)*	Sulfate (mg/L)	Sulfide (mg/L)	Methane (mg/L)	Total Iron (unfiltered) (mg/L)	Dissolved Iron* (filtered) (mg/L)
BW01	11/20/13	2,800	250	<0.3	<0.15	6.7	<2.0	3.6	87	43
BW01	03/05/14	60	130	<0.06	<0.03	98	<2.0	5.0	31	15
BW01	06/05/14	420	23	<0.3	<0.15	32	<2.0	13	49	37
BW01	09/24/14	1,000	57	<1.2	<0.6	<6.0	<2.0	9.0	140	100
BW01	12/18/14	1,300	64	na	na	9.6	<2.0	11	190	150
BW01	03/25/15	34	4.0	na	na	74	<2.0	3.5	14	14
BW01	06/25/15	0	4.7	na	na	53	na	5.9	16	16
BW01	09/29/15	980	74	na	na	2.4	na	9.0	170	120
BW01	01/20/16	50	6.4	na	na	83	na	9.3	22	22
BW01	04/08/16	350	3.9	na	na	60	na	4.4	10	10
BW01	06/24/16	250	13	na	na	24	na	14.0	45	42
BW01	09/21/16	680	33	na	na	2.7	na	9.1	120	140
BW02	11/20/13	2,400	220	<0.3	<0.15	1.9	<2.0	9.2	110	83
BW02	03/05/14	730	23	<0.06	<0.03	3.5	<2.0	17	110	88
BW02	06/05/14	880	120	<0.3	<0.15	<1.5	<2.0	13	110	89
BW02	09/24/14	1,500	120	<1.2	<0.6	<6.0	<2.0	9.3	190	160
BW02	12/18/14	520	75	na	na	26	<2.0	13	150	140
BW02	03/25/15	510	75	na	na	4.1	<2.0	13	100	100
BW02	06/25/15	1,100	93	na	na	<0.6	na	12	220	190
BW02	09/29/15	1,300	110	na	na	3.9	na	9.4	210	160
BW02	01/20/16	540	55	na	na	3.5	na	19	130	120
BW02	04/08/16	810	65	na	na	<0.3	na	16	180	170
BW02	06/24/16	410	46	na	na	3.5	na	13	94	91
BW02	09/21/16	640	50	na	na	2	na	11	150	160
BW03	11/20/13	2,800	260	<0.3	<0.15	<0.3	<2.0	9.1	83	51
BW03	03/05/14	3,100	260	<0.3	<0.15	<1.5	<2.0	13	100	41
BW03	06/05/14	3,100	270	<0.3	<0.15	<1.5	<2.0	11	130	79
BW03	09/24/14	2,700	250	<3.0	<1.5	<1.5	<2.0	9.6	150	89
BW03	12/18/14	1,400	92	na	na	45	<2.0	13	85	54
BW03	03/25/15	2,500	190	na	na	1.1	<2.0	12	140	93
BW03	06/25/15	2,200	160	na	na	<0.6	na	6.6	120	69
BW03	09/29/15	1,900	180	na	na	1.3	na	8.6	170	80
BW03	01/15/16	1,500	110	na	na	3.5	na	15	120	20
BW03	04/08/16	2,000	140	na	na	1.9	na	13	150	130
BW03	06/24/16	1,200	150	na	na	<0.3	na	12	170	120
BW03	09/21/16	1,600	140	na	na	1.1	na	13	150	150
BW04	11/20/13	2,800	120	<0.3	<0.15	11	<2.0	9.6	71	40
BW04	03/05/14	1500	110	<0.12	<0.06	5.3	<2.0	14	70	41
BW04	06/05/14	970	65	<0.3	<0.15	<1.5	<2.0	13	62	38
BW04	09/24/14	1,700	150	<.0015	<0.75	<7.5	<2.0	11	160	98
BW04	12/18/14	640	35	na	na	43	<2.0	14	93	64
BW04	03/25/15	330	16	na	na	1.7	<2.0	14	56	39
BW04	06/25/15	410	21	na	na	<5.9	na	10	74	39
BW04	09/29/15	440	43	na	na	1.2	na	12	110	29
BW04	01/15/16	290	14	na	na	3.6	na	17	80	0.1
BW04	04/08/16	220	9.7	na	na	1.1	na	12	62	55
BW04	06/24/16	220	8.8	na	na	1.8	na	12	70	66
BW04	09/21/16	410	22	na	na	2.3	na	12	120	110
BW05	11/20/13	2,900	350	<0.3	<0.15	<0.3	<2.0	10	110	65
BW05	03/05/14	2,600	290	<0.12	<1.5	<0.6	<2.0	12	140	81
BW05	06/05/14	2,600	280	<0.3	<0.15	<1.5	<2.0	9.8	150	100
BW05	09/24/14	2,500	270	<.0003	<0.15	<1.5	<2.0	8.7	160	95
BW05	12/18/14	1,900	190	na	na	5.6	<2.0	11	160	120
BW05	03/25/15	1,800	170	na	na	7.7	<2.0	12	140	120
BW05	06/25/15	1,700	160	na	na	<4.9	na	14	150	99
BW05	09/29/15	1,400	150	na	na	1.2	na	12	150	69
BW05	01/15/16	1,600	130	na	na	2.5	na	17	170	82
BW05	04/08/16	1,200	90	na	na	2.7	na	11	150	97
BW05	06/24/16	1,500	130	na	na	<0.3	na	8.9	190	150
BW05	09/21/16	1,400	120	na	na	2.2	na	12	180	160
BW06	11/20/13	880	95	<0.06	<0.15	74	<2.0	7.7	88	87
BW06	03/05/14	870	98	<0.06	<0.15	75	<2.0	6.0	120	84
BW06	06/05/14	1,700	110	<0.12	<0.75	25	<2.0	12	100	75
BW06	09/24/14	1,700	110	<0.12	<0.75	25	<2.0	12	120	86
BW06	12/18/14	1,300	90	<0.12	<0.06	31	<2.0	11	140	120
BW06	03/24/15	1,300	88	<0.12	<0.07	29	<2.0	13	95	63
BW06	06/25/15	700	36	na	na	89	na	14	100	75
BW06	09/28/15	750	51	na	na	33	na	11	120	64
BW06	01/15/16	550	32	na	na	110	na	18	110	24
BW06	04/08/16	750	31	na	na	99	na	15	110	97
BW06	06/24/16	600	33	na	na	100	na	13	95	94
BW06	09/21/16	590	40	na	na	95	na	12	110	110
BW07	11/20/13	2,300	240	<0.06	<0.15	<0.3	<2.0	6.9	140	97
BW07	03/05/14	2,400	250	<0.12	<1.5	5.8	<2.0	13	110	79
BW07	06/05/14	2,200	240	<0.3	<0.15	<1.5	<2.0	12	140	120
BW07	09/24/14	2,400	260	<3.0	<0.15	<1.5	<2.0	8	180	140
BW07	12/18/14	1,700	150	na	na	27	<2.0	12	150	87
BW07	03/24/15	1,700	130	na	na	12	<2.0	14	120	84
BW07	06/25/15	1,800	150	na	na	<0.6	na	12	140	120
BW07	09/28/15	1,800	220	na	na	na	na	6.8	180	130
BW07	01/15/16	1,400	110	na	na	8.1	na	15	150	84
BW07	04/08/16	1,200	89	na	na	31	na	9.6	150	150
BW07	06/24/16	1,500	120	na	na	1.9	na	11	160	150
BW07	09/21/16	2,000	200	na	na	1.4	na	8.9	180	180
BW08	11/20/13	2,600	230	<0.06	<0.15	<0.3	<2.0	7.6	140	120
BW08	03/05/14	2,000	210	<0.12	<0.06	6.1	<2.0	17	110	62
BW08	06/05/14	2,500	230	<0.3	<0.15	<1.5	<2.0	10	150	75
BW08	09/24/14	2,100	230	<3.0	<0.15	<1.5	<2.0	7.7	190	130
BW08	12/18/14	800	69	na	na	69	<2.0	12	110	78
BW08	03/24/15	820	62	na	na	87	<2.0	14	81	50
BW08	06/25/15	2,700	47	na	na	63	na	6.3	110	69
BW08	09/28/15	1,300	85	na	na	<0.3	na	7.4	170	120
BW08	01/15/16	210	18	na	na	110	na	13	66	0.081
BW08	04/08/16	190	15	na	na	93	na	6.7	55	50
BW08	06/23/16	440	35	na	na	57	na	10	84	73
BW08	09/21/16	1,100	79	na	na	1.5	na	9.1	160	160
BW09	11/20/13	380	32	<0.06	<0.15	78	<2.0	7.0	42	40
BW09	03/05/14	291	24	<0.06	<0.03	59	<2.0	14	49	28
BW09	06/05/14	360	33	<0.06	<0.03	10	<2.0	12	78	45
BW09	09/24/14	640	39	<0.6	<0.3	42	<2.0	6.7	88	62
BW09	12/18/14	390	20	na	na	48	<2.0	12	120	100
BW09	03/24/15	160	13	na	na	45	<2.0	13	57	42
BW09	06/25/15	320	19	na	na	16	na	6.6	110	81
BW09	09/28/15	410	23	na	na	20	na	7.2	100	66
BW09	01/15/16	190	14	na	na	20	na	18	91	24
BW09	04/08/16	140	9.8	na	na	30	na	14	70	65
BW09	06/23/16	380	18	na	na	22	na	10	97	93
BW09	09/21/16	500	26	na	na	9.6	na	9.3	150	150
BW10	11/20/13	1,700	200	<0.06	<0.15	<0.3	<2.0	9.1	120	96
BW10	03/05/14	1,300	130	<0.12	<0.06	8.6	<2.0	18	84	41
BW10	06/04/14	830	77	<0.06	<0.03	2.8	<2.0	13	83	40
BW10	09/24/14	1,200	85	<1.2	<0.6	<6.0	<2.0	7.0	130	87
BW10	12/18/14	1,200	48	na	na	49	<2.0	11	31	19
BW10	03/23/15	420	25	na	na	22	<2.0	14	63	41
BW10	06/25/15	550	35	na	na	4.1	na	8.2	100	100
BW10	09/28/15	970	69	na	na	2.3	na	8.2	150	77
BW10	01/15/16	650	39	na	na	<0.3	na	18	140	79
BW10	04/08/16	410	30	na	na	15	na	15	76	71
BW10	06/23/16	590	37	na	na	<0.3	na	13	100	99
MW06**	09/21/16	35	1.8	na	na	250	na	110	27	19

Appendix A13: Volatile organic compound (VOC) concentrations measured in the biowall wells (BWs). Blank values indicate that the compound was not detected.

Well	Sample Date	TCE (µg/L)	cis-1,2-DCE (µg/L)	trans-1,2-DCE (µg/L)	1,1-DCE (µg/L)	VC (µg/L)	Ethene (µg/L)
BW1	Nov-13			10			
	Mar-14	2.4					
	Jun-14	0.46					
	Sep-14						
	Dec-14						
	Mar-15	0.62					
	Jun-15						
	Sep-15		0.66				
	Jan-16						
	Apr-16	0.51					
	Jun-16	0.32	0.63				
	Sep-16		0.43				
BW2	Nov-13						
	Mar-14						
	Jun-14						
	Sep-14						
	Dec-14						
	Mar-15						
	Jun-15						
	Sep-15						
	Jan-16						
	Apr-16						
	Jun-16						
	Sep-16						
BW3	Nov-13						
	Mar-14						
	Jun-14						
	Sep-14						
	Dec-14						
	Mar-15						
	Jun-15						
	Sep-15						
	Jan-16						
	Apr-16						
	Jun-16						
	Sep-16						1.1
BW4	Nov-13						
	Mar-14						
	Jun-14						
	Sep-14						
	Dec-14						
	Mar-15						
	Jun-15						
	Sep-15						
	Jan-16						
	Apr-16		0.36				
	Jun-16		0.34				
	Sep-16						
BW5	Nov-13						
	Mar-14						
	Jun-14						
	Sep-14						
	Dec-14						
	Mar-15						
	Jun-15		0.32				
	Sep-15						
	Jan-16						
	Apr-16		0.57				
	Jun-16						
	Sep-16						

Well	Sample Date	TCE (µg/L)	cis-1,2-DCE (µg/L)	trans-1,2-DCE (µg/L)	1,1-DCE (µg/L)	VC (µg/L)	Ethene (µg/L)
BW 6	Nov-13	9.1	250	1.3		0.96	
	Mar-14	1.6	140	1.2		14	
	Jun-14	0.7	100	0.66		34	1.2
	Sep-14		75	0.31		44	4.2
	Dec-14	0.33	78	0.33		21	1.9
	Mar-15	25	150	1.0	4.7	25	4.4
	Jun-15	1.4	160	0.71	3.3	20	6.9
	Sep-15		66		0.73	25	4.0
	Jan-16	11	230	0.65	2	13	4.7
	Apr-16	36	180	0.54	1.5	9.6	5.3
	Jun-16	0.68	230	0.51	1.3	11	7.2
	Sep-16		110			12	4.6
BW 7	Nov-13		31	0.37		0.36	
	Mar-14		8.2			1.2	
	Jun-14		16			2.2	
	Sep-14	0.79	21			10	1.0
	Dec-14		5.3			9.0	1.2
	Mar-15	1.1	4.7			8.7	2.0
	Jun-15					1.4	2.0
	Sep-15					0.47	
	Jan-16					0.33	1.0
	Apr-16	0.47	7.1	0.33		1.3	
	Jun-16		0.68			0.33	0.3
	Sep-16	0.32					
BW 8	Nov-13		31	0.32		0.34	
	Mar-14		50	0.55		7.3	1.3
	Jun-14		16	0.36		11	1.8
	Sep-14		0.44			2.3	1.7
	Dec-14	34	28		3.6	10	1.4
	Mar-15	7.3	12	0.41	0.84	4.2	3.7
	Jun-15	2.1	24		0.69	8.3	1.9
	Sep-15						
	Jan-16	41	65	0.55	1.3	16	4.4
	Apr-16	23	54	0.45	0.44	13	4.6
	Jun-16		42			10	7.2
	Sep-16	0.4	0.32				
BW 9	Nov-13		3.0				
	Mar-14	1.1					
	Jun-14	1.0	0.89				
	Sep-14	0.47	3.4				
	Dec-14		0.56				
	Mar-15	1.0	0.32				
	Jun-15	0.4	1.0				
	Sep-15		6.2				
	Jan-16	2.7	0.93				
	Apr-16	2.2	0.85				
	Jun-16	0.72	1.7				
	Sep-16	0.61	4.4				
BW 10	Nov-13						
	Mar-14						
	Jun-14						
	Sep-14						
	Dec-14						
	Mar-15						
	Jun-15						
	Sep-15						
	Jan-16						
	Apr-16						
	Jun-16						

Appendix A14: Volatile organic compound (VOC) concentrations measured in the Remedial investigation wells (MWs) and Transect wells (TW). Blank values indicate that the compound was not detected.

Well	Sample Date	TCE (µg/L)	cis-1,2-DCE (µg/L)	trans-1,2-DCE (µg/L)	1,1-DCE (µg/L)	VC (µg/L)	Ethene (µg/L)
TW6	Nov-13	470	10				na
	Mar-14	660	4.0		0.99		na
	Jun-14	590	8.0		0.93		na
	Sep-14	500	9.0		0.75		na
	Dec-14	530	10		0.83		
	Mar-15	1,100	7.6		1.1		
	Jun-15	390	11		0.96		
	Sep-15	420	35	0.39	1.0		
	Jan-16	580	7.2				
	Apr-16	550	9.5		1.0		
	Jun-16	600	9.7		1.1		
	Sep-16	580	8.8				
TW5	Nov-13	450	9.9				na
	Mar-14	660	8.5	0.33	0.97		na
	Jun-14	540	11		0.98		na
	Sep-14	470	32	0.32	0.84	2.4	na
	Dec-14	510	20		0.81	0.97	
	Mar-15	1,100	26		1.1		
	Jun-15	410	16		1.2		
	Sep-15	400	29		1.2	2.0	1.5
	Jan-16	590	7.5		1.1		
	Apr-16	530	19	0.35	1.1		
	Jun-16	610	26	0.43	1.1		
	Sep-16	540	25				
TW4	Nov-13	44	92			0.69	na
	Mar-14	240	120	0.76	0.58	9.9	na
	Jun-14	380	110	0.51	0.96	25	na
	Sep-14	200	130	0.38	0.52	38	na
	Dec-14	160	64		0.41	24	3.5
	Mar-15	340	92	0.34	0.96	16	4.1
	Jun-15	280	98	0.42	0.99	16	3.6
	Sep-15	71	140	0.35	0.42	15	4.9
	Jan-16	300	120	0.39	0.82	9.1	4.3
	Apr-16	340	120	0.4	0.93	6.8	4.1
	Jun-16	260	150	0.43	0.7	9	7.5
	Sep-16	220	150			8.8	5.8
TW3	Nov-13	0.82	88			0.34	na
	Mar-14		62	0.58		7.5	na
	Jun-14		54	0.39		31	na
	Sep-14	0.39	39			40	na
	Dec-14	1.6	12			36	4.2
	Mar-15	2.3	31		0.33	9.8	5.6
	Jun-15		36			9.8	5.3
	Sep-15		1.6			1.5	2.6
	Jan-16	3.5	33		0.82	6.7	4.4
	Apr-16	3.3	35			5.8	4.6
	Jun-16	13	40			8.3	7.4
	Sep-16	3.6	31			5.9	3.6
TW2	Nov-13	64	240				na
	Mar-14	4.2	200	1.2	0.37	13	na
	Jun-14	2.2	200	1.1	2.4	46	na
	Sep-14		150		0.74	56	na
	Dec-14	0.71	170	0.39	0.67	46	3.9
	Mar-15	9.5	210	0.96	4.3	26	4.5
	Jun-15		220	0.85	4.1	26	3.6
	Sep-15		160	0.38	2.4	32	7.2
	Jan-16	5.1	280	0.8	3.1	20	4.4
	Apr-16	29	260	0.73	2.6	15	5.0
	Jun-16		250	0.47	2	19	5.7
	Sep-16		190			21	6
TW1	Nov-13	71	260				na
	Mar-14	7.5	180	1.3	0.31	13	na
	Jun-14	2.2	200	1.0	2.2	45	na
	Sep-14	0.34	160		0.70	56	na
	Dec-14		160	0.34	0.54	46	4.0
	Mar-15	8.6	210	1.0	4.3	27	4.7
	Jun-15	0.48	240	1.0	4.7	27	2.6
	Sep-15		240		2.7	33	6.7
	Jan-16	5.4	130	0.71	2.9	18	4.3
	Apr-16	21	250	0.71	2.5	15	4.6
	Jun-16		230	0.52	2.1	19	5
	Sep-16		200			23	4.8
TW0	Jun-16	4.3	270	0.55	2.3	19	5.5
	Sep-16		270			23	4.8

Well	Sample Date	TCE (µg/L)	cis-1,2-DCE (µg/L)	trans-1,2-DCE (µg/L)	1,1-DCE (µg/L)	VC (µg/L)	Ethene (µg/L)
MW1	Mar-14						na
	Sep-14						na
	Mar-15						na
	Sep-15						na
	Apr-16						na
	Sep-16	0.85					na
MW2	Mar-14	41	4.6				na
	Sep-14	46	13			0.60	na
	Mar-15	42	3.7				na
	Sep-15	41	15			0.79	na
	Apr-16	47	4.3				na
	Sep-16	19	22			1.1	na
MW3	Mar-14						na
	Sep-14						na
	Mar-15						na
	Sep-15						na
	Apr-16						na
	Sep-16						na
MW4	Mar-14						na
	Sep-14						na
	Mar-15						na
	Sep-15						na
	Apr-16						na
	Sep-16						na
MW5	Mar-14						na
	Sep-14						na
	Mar-15						na
	Sep-15						na
	Apr-16						na
	Sep-16						na
MW6	Mar-14	430	2.8		0.79		na
	Sep-14	510	5.5		0.81		na
	Mar-15	410	2.5		0.61		na
	Sep-15	420	12		0.84		na
	Apr-16	410	8.7		0.64		na
	Sep-16	580					na
MW7	Mar-14						na
	Sep-14						na
	Mar-15						na
	Sep-15						na
	Apr-16						na
	Sep-16	0.36					na

Appendix B1: GC method iterations

Method 19 1 µL injection
oven 70 - 90°C @ 4°C/min, hold 2 min; 90 - 190°C @ 45°C/min, hold 2 min

Method 21 1 µL injection
inlet: 200°C @ 3mL/min; 10:1 split
250°C; H_2 = 40, Air = 450, N_2 =
FID: 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-230 @ 45°C/min, hold 2 min

Method 21-2 1 µL injection
inlet: 200°C @ 3mL/min; 10:1 split
250°C; H_2 = 35, Air = 350, N_2 =
FID: 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-230 @ 45°C/min, hold 2 min

Method 21-3 1 µL injection
inlet: 200°C @ 3mL/min; 10:1 split
280°C; H_2 = 35, Air = 350, N_2 =
FID: 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

Method 21-4 1 µL injection
inlet: 200°C @ 3mL/min; 10:1 split
280°C; H_2 = 40, Air = 350, N_2 =
FID: 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min
**for 0703 and 0704seq, mid temp for oven was 65 not 70°C; changed to 70°C for 0708seq

Method 21-4b 1 µL injection
inlet: 200°C @ 3mL/min; 5:1 split
280°C; H_2 = 40, Air = 350, N_2 =
FID: 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min; 90-250 @ 60°C/min.

Method 21-4c 1 µL injection
inlet: 200°C @ 3mL/min; 5:1 split
280°C; H_2 = 40, Air = 350, N_2 =
FID: 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

Method 21-4d 1 µL injection
inlet: 200°C @ 3mL/min; 100:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

Method 21-5 1 µL injection
inlet: 200°C @ 3mL/min; 10:1 split
FID: 250°C; H_2 = 40, Air = 350, N_2 = 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-230 @ 45°C/min, hold 2 min

Method 22-4 2 µL injection
inlet: 200°C @ 3mL/min; 10:1 split
FID: 280°C; H_2 = 40, Air = 350, N_2 = 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

Method 22-4b 2 µL injection
inlet: 200°C @ 3mL/min; 100:1 split
FID: 280°C; H_2 = 40, Air = 350, N_2 = 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

Clean MeOH 1 µL injection
inlet: 220°C @ 3mL/min; splitless
FID: 330°C; H_2 = 45, Air = 380, N_2 = 35
oven: 40-280 @ 10°C/min, hold 240 min

Method 0701SPLITLESS 1 µL injection
inlet: 180°C @ 1.5mL/min; splitless
FID: 280°C; H_2 = 35, Air = 350, N_2 = 30
oven: 40-90 @ 5°C/min, hold 1 min; 90-230 @ 45°C/min, hold 2 min

TCE Method 1 1 µL injection
inlet: 250°C @ 1.0mL/min; 100:1 split
FID: 280°C; H_2 = 30, Air = 400, N_2 = 25
oven: 50°C, hold 4 min; ramp to 260°C @ 6°C/min, hold 8 min

TCE Method 2 1 µL injection
inlet: 200°C @ 3.0mL/min; 5:1 split
FID: 280°C; H_2 = 40, Air = 350, N_2 = 30
oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

TCE Method 3 1 µL injection

inlet: 200°C @ 3.0mL/min; 5:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30

oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

TCE Method 4 1 µL injection

inlet: 200°C @ 3.0mL/min; 5:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30

oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

TCE Method 5 1 µL injection

inlet: 200°C @ 3.0mL/min; 5:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30

oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

TCE Method 6 1 µL injection

inlet: 200°C @ 3.0mL/min; 5:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30

oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

TCE Method 7 1 µL injection

inlet: 200°C @ 3.0mL/min; 5:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30

oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

TCE Method 8 1 µL injection

inlet: 200°C @ 3.0mL/min; 5:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30

oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

TCE Method 9 1 µL injection

inlet: 200°C @ 3.0mL/min; 5:1 split

FID: 280°C; H_2 = 40, Air = 350, N_2 = 30

oven: 40-70 @ 20°C/min, hold 1 min; 70-90 @ 5°C/min, hold 2 min; 90-250 @ 45°C/min, hold 2 min

Appendix B2: Raw ICP data

Sample Name	G #	Al Quant [1] ppm	Al Quant [2] ppm	Al Quant [3] ppm	Al Quant Average ppm	Ca Quant [1] ppm	Ca Quant [2] ppm	Ca Quant [3] ppm	Quant Average ppm	Cd Quant [1] ppb	Cd Quant [2] ppb	Cd Quant [3] ppb	Quant Average ppb	Co Quant [1] ppb	Co Quant [2] ppb	Co Quant [3] ppb	Quant Average ppb	Fe Quant [1] ppb	Fe Quant [2] ppb	Fe Quant [3] ppb	Fe Quant Average ppb
Reactor001	1	0.137	0.139	0.148	0.141 L	67.4	70	69.6	69	12.1	10.6	10.5	11	-2.39	-2.69	-2.18	-2.42 L	6070	6150	6230	6150
Reactor002	1	-0.0648	-0.0734	-0.061	-0.0664 L	65.8	65.8	65.9	65.8	8.38	8.14	7.99	8.17	-2.81	-2.07	-3.01	-2.63 L	5950	5930	5880	5920
Reactor003	1	0.404	0.391	0.404	0.4	46.2	46.6	46.7	46.5	7.74	7.92	8.29	7.98	-1.8	-1.83	-1.83	-1.82 L	6940	7060	7020	7010
Reactor005	1	-0.497	-0.504	-0.498	-0.499 L	274	275	275	275	34.1	34.1	35.2	34.5 L	21.3	22	21.4	21.6 L	382000	384000	386000	384000
Reactor006	1	-0.275	-0.285	-0.28	-0.280 L	70.9	71.6	71.3	71.3	9.26	8.08	7.92	8.42	-5.42	-4.89	-5.7	-5.34 L	1710	1620	1650	1660 L
Reactor007	1	3.07	3.1	3.09	3.09	65.3	65.6	65.6	65.5	11.8	12.2	12.4	12.1	-5.42	-5.84	-5.72	-5.66 L	64300	64600	64600	64500
Reactor008	1	-0.226	-0.228	-0.249	-0.234 L	50	50.2	50.7	50.3	8.26	8.56	8.43	8.42	-5.07	-5.53	-5.72	-5.44 L	6770	6840	6860	6820
Reactor009	1	0.371	0.355	0.368	0.365	7.35	7.46	7.49	7.44	7.82	8.22	8.39	8.14	0.409	0.574	0.927	0.637 L	1150	1170	1170	1170 L
Reactor010	1	-0.124	-0.144	-0.127	-0.132	-2.52	-2.48	-2.49	-2.5	9.16	8.92	8.7	8.93	6.3	5.03	5.69	5.67 L	7280	7450	7440	7390
Reactor011	1	0.111	0.147	0.144	0.134 L	372	373	375	373	11.4	10.4	10.7	10.9	-5.73	-4.14	-5.06	-4.98 L	40900	41300	41600	41300
Reactor012	1	0.57	0.595	0.671	0.612 L	258	262	266	262	11.7	12.4	11.9	12.0 L	-8.58	-10	-9.62	-9.41 L	52500	53600	54000	53300
Reactor013	1	0.336	0.381	0.407	0.375 L	219	222	223	221	10.8	10.5	11.1	10.8	-12.5	-12	-12.7	-12.4 L	37700	38100	38400	38100
Reactor014	1	1.6	1.6	1.59	1.60 L	207	209	209	208	26.5	26.3	26.8	26.5	16.9	17.6	18.3	17.6 L	242000	244000	244000	243000
Reactor016	1	0.426	0.483	0.474	0.461 L	305	310	314	310	9.83	10.1	9.77	9.91	-15.3	-15	-14.4	-14.9 L	5210	5170	5250	5210
Reactor017	1	2.99	3.13	3.19	3.1	139	144	148	144	15.8	16.8	17	16.6	-15.9	-15.7	-16.3	-16.0 L	83100	85800	87600	85500
Reactor018	1	2.54	2.73	2.72	2.66	158	160	163	160	12.1	12	12.6	12.2	-7.67	-7.63	-7.83	-7.71 L	44100	44700	45500	44800
Reactor019	1	1.44	1.59	1.79	1.61	57.5	58.3	59.8	58.5	7.63	7.03	7.2	7.29	0.884	1	1.68	1.19 L	2520	2410	2490	2470
Reactor020	1	-0.421	-0.421	-0.407	-0.416 L	3.77	4.25	4.91	4.31	8.01	8.03	8.5	8.18	119	125	134	126	5260	5440	5820	5510

Sample Name	K Quant [1] ppm	K Quant [2] ppm	K Quant [3] ppm	K Quant Average ppm	Mg Quant [1] ppm	Mg Quant [2] ppm	Mg Quant [3] ppm	Quant Average ppm	P Quant [1] ppm	P Quant [2] ppm	P Quant [3] ppm	P Quant Average ppm	S Quant [1] mg/L	S Quant [2] mg/L	S Quant [3] mg/L	S Quant Average mg/L	Si Quant [1] ppb	Si Quant [2] ppb	Si Quant [3] ppb	Si Quant Average ppb
Reactor001	9.85	9.85	9.99	9.9	10.4	10.5	10.6	10.5	0.704	0.689	0.709	0.701	1.17	1.23	1.23	1.21	3520	3580	3610	3570
Reactor002	10.3	10.3	10.2	10.3	10.5	10.5	10.5	10.5	0.444	0.455	0.467	0.456	1.16	1.16	1.18	1.16	2350	2350	2370	2360
Reactor003	9.13	9.17	9.07	9.13	8.4	8.48	8.39	8.42	0.439	0.458	0.458	0.452	0.969	0.944	0.971	0.961	2300	2320	2330	2320
Reactor005	13.6	13.6	13.4	13.5	25.3	25.3	25.2	25.3	0.223	0.2	0.229	0.218	3.42	3.43	3.47	3.44	924	933	943	933
Reactor006	15.5	15.5	15.3	15.4	15.3	15.3	15.2	15.3	1.93	1.96	1.97	1.95	1.65	1.68	1.7	1.68	2770	2800	2790	2780
Reactor007	14.8	14.6	14.5	14.6	14.8	14.7	14.7	14.7	3.39	3.41	3.44	3.41	2.37	2.39	2.4	2.38	9370	9410	9420	9400
Reactor008	17	16.8	17	16.9	14.2	14.2	14.3	14.2	0.449	0.471	0.438	0.453	1.2	1.17	1.17	1.18	1220	1240	1260	1240
Reactor009	2.41	2.4	2.41	2.41	2.83	2.85	2.85	2.84	0.0679	0.0396	0.0463	0.0513 L	2.78	2.8	2.78	2.79	5460	5520	5530	5500
Reactor010	2.2	2.2	2.2	2.2	1.52	1.53	1.53	1.52	0.0624	0.0455	0.0484	0.0521 L	1.6	1.63	1.63	1.62	-206	-201	-199	-202
Reactor011	47.5	47.9	48.2	47.9	57.7	58.1	58.3	58	2.88	2.92	2.94	2.91	5.34	5.35	5.41	5.37	14400	14500	14500	14500
Reactor012	45.7	46.1	47.3	46.4	47.8	48.4	49.1	48.5	1.21	1.23	1.25	1.23	3.69	3.78	3.83	3.77	12600	12800	13000	12800
Reactor013	42.1	43.3	43.7	43	40.8	41.4	41.7	41.3	0.866	0.871	0.88	0.872	3.34	3.39	3.41	3.38	7670	7780	7790	7750
Reactor014	40.3	40.3	41.1	40.6	39.2	39.3	39.4	39.3	0.623	0.61	0.623	0.619	2.71	2.73	2.69	2.71	16500	16500	16500	16500
Reactor016	79.7	82	84.3	82	70.1	71.4	72.6	71.3	8.99	9.18	9.33	9.17	27.4	27.8	28.1	27.7	13700	13900	14000	13900
Reactor017	66.3	69.6	72.8	69.6	45.2	46.8	48.4	46.8	3.62	3.74	3.85	3.73	3.82	3.95	4.04	3.94	13300	13600	14100	13600
Reactor018	71.9	72.8	75	73.3	43.2	43.8	44.7	43.9	4.43	4.5	4.61	4.51	3.5	3.56	3.61	3.56	7610	7710	7860	7730
Reactor019	3.81	3.84	3.91	3.85	5.13	5.21	5.38	5.24	0.106	0.0969	0.102	0.102 L	14.8	15.1	15.4	15.1	11400	11500	12000	11700
Reactor020	2.61	2.65	2.68	2.64	3.31	3.45	3.65	3.47	0.0402	0.051	0.0348	0.0420 L	10.8	11.5	12.2	11.5	278	332	405	338

Appendix B3: Cleaned ICP data

Reactor/Analyte	Ca (ppm)	Cd (ppb)	Co (ppb)	Fe (ppm)	Group 1 K (ppm)	Mg (ppm)	P (ppm)	S (mg/L)	Si (ppm)
M4/C3: G0 + Fe0	690.0	110.0	-	61.5	99.0	105.0	7.0	12.1	35.7
M4/C3: G0 + Fe5	658.0	81.7	-	59.2	103.0	105.0	4.6	11.6	23.6
M4/C3: G0 + Fe10	465.0	79.8	-	70.1	91.3	84.2	4.5	9.6	23.2
M4C3: G10 + Fe10	-	-	-	-	-	-	-	-	-
M4/C3: G30 + Fe10	2750.0	345.0	216.0	4150.0	135.0	253.0	2.2	34.4	9.3
M1/C1: G0 + Fe0	713.0	84.2	-	16.6	154.0	153.0	19.5	16.8	27.8
M1/C1: G10 + Fe10	655.0	121.0	-	645.0	146.0	147.0	34.1	23.8	94.0
M1/C1: G0 + Fe10	503.0	84.2	-	68.2	169.0	142.0	4.5	11.8	12.4
Sand Spike	74.4	81.4	6.37	11.7	24.1	28.4	0.513	27.9	55.0
Soil Spike	-	89.3	56.7	73.9	22.0	15.2	0.521	16.2	-

Reactor/Analyte	Ca (ppm)	Cd (ppb)	Co (ppb)	Fe (ppm)	Group 2 K (ppm)	Mg (ppm)	P (ppm)	S (mg/L)	Si (ppm)
M4/C3: G0 + Fe0	746.0	21.8	-	82.6	95.8	116.0	5.8	10.7	29.0
M4/C3: G0 + Fe5	524.0	24.0	-	106.6	92.8	97.0	2.5	7.5	25.6
M4/C3: G0 + Fe10	442.0	21.6	-	76.2	86.0	82.6	1.7	6.8	15.5
M4C3: G10 + Fe10	416.0	53.0	35.2	486.0	81.2	78.6	1.2	5.4	33.0
M4/C3: G30 + Fe10	-	-	-	-	-	-	-	-	-
M1/C1: G0 + Fe0	620.0	19.8	-	10.4	164.0	142.6	18.3	55.4	27.8
M1/C1: G10 + Fe10	288.0	33.2	-	171.0	139.2	93.6	7.5	7.9	27.2
M1/C1: G0 + Fe10	320.0	24.4	-	89.6	146.6	87.8	9.0	7.1	15.5
Sand Spike	117.0	14.6	2.4	4.9	7.7	10.5	0.204	30.2	23.4
Soil Spike	8.6	16.4	252.0	11.0	5.3	6.9	0.084	23.0	0.676

Appendix B4: Total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) content of the batch reactors, groups 1 and 2

TC	TC Conc (%)					Wt (g)					TC (g)				
Row Labels	**1**	**2**	**3**	*Avg*	*SD*	**1**	**2**	**3**	*Avg*	*SD*	**1**	**2**	**3**	*Avg*	*SD*
1	4.89	5.98	4.17	*5.01*	*0.91*	100.6	99.7	99.7	*100.0*	*0.5*	4.92	5.96	4.15	*5.01*	*0.91*
2	7.55	9.81	7.69	*8.35*	*1.27*	99.3	100.5	102.6	*100.8*	*1.7*	7.50	9.86	7.89	*8.41*	*1.26*
3	15.06	10.78	10.55	*####*	*0.16*	99.8	99.1	101	*100.0*	*1.0*	15.03	10.68	10.66	*10.67*	*0.02*
5	7.87	12.71	6.71	*7.29*	*0.82*	101.1	100.5	100.6	*100.7*	*0.3*	7.95	12.77	6.75	*7.35*	*0.85*
6	2.73	1.97	2.24	*2.31*	*0.38*	102.9	104.9	100.6	*102.8*	*2.2*	2.80	2.07	2.26	*2.38*	*0.38*
7	4.52	4.12	5.07	*4.57*	*0.48*	100.6	101.8	101.1	*101.2*	*0.6*	4.55	4.19	5.13	*4.62*	*0.47*
8	1.99	2.25	2.37	*2.20*	*0.19*	100.6	99.5	104.7	*101.6*	*2.7*	2.00	2.24	2.48	*2.24*	*0.24*
9	0.08			*0.08*		101.9			*101.9*		0.08			*0.08*	
10	0.32	0.40	0.40	*0.37*	*0.04*	102.7	101.7	101.5	*102.0*	*0.6*	0.33	0.40	0.41	*0.38*	*0.04*
11	9.94	12.30	8.89	*####*	*1.75*	103.9	101.9	103.2	*103.0*	*1.0*	10.32	12.53	9.17	*10.68*	*1.71*
12	5.96	10.31	12.58	*####*	*1.61*	101.4	101.6	101.8	*101.6*	*0.2*	6.04	10.47	12.81	*11.64*	*1.65*
13	6.62	7.74	7.10	*7.15*	*0.56*	104.9	102.2	103	*103.4*	*1.4*	6.95	7.91	7.32	*7.39*	*0.49*
14	24.85	10.59	4.83	*7.71*	*4.08*	106.3	101	102.4	*103.2*	*2.7*	26.42	10.70	4.94	*7.82*	*4.07*
16	3.74	3.70	4.40	*3.95*	*0.39*	99.8	105.9	100.6	*102.1*	*3.3*	3.73	3.92	4.42	*4.03*	*0.36*
17	4.07	3.78	5.19	*4.34*	*0.74*	100.2	103.8	101.3	*101.8*	*1.8*	4.08	3.92	5.25	*4.42*	*0.73*
18	3.25	3.18	4.41	*3.61*	*0.69*	100.2	101.7	103	*101.6*	*1.4*	3.25	3.24	4.54	*3.68*	*0.75*
19	0.07			*0.07*		100.7			*100.7*		0.07			*0.07*	
20	0.28	0.27	0.29	*0.28*	*0.01*	99.1	101.3	102.8	*101.1*	*1.9*	0.27	0.28	0.30	*0.29*	*0.02*
M4C3	3.50	5.86		*4.68*	*1.67*	101.5	95.4		*98.5*	*4.3*	3.55	5.59	0.00	*3.05*	*2.83*

	TC (%)		Wt (g)		TC (g)		IC (%)		Wt (g)		TOC	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	%	fOC
1	5	0.9	100.0	0.5	5.01	0.91	0.05	0.03	500.6	0.8	4.96	0.05
2	8.3	1.3	100.8	1.7	8.41	1.26	0.08	0.03	504.3	4.4	8.26	0.08
3	11	0.2	100.0	1.0	10.67	0.02	0.06	0.00	503.5	3.0	####	0.11
5	7.3	0.8	100.7	0.3	7.35	0.85	0.01		504.4	0.9	7.27	0.07
6	2.3	0.4	102.8	2.2	2.38	0.38	0.03	0.02	502.6	1.7	2.29	0.02
7	4.6	0.5	101.2	0.6	4.62	0.47	0.16	0.02	501.3	1.6	4.41	0.04
8	2.2	0.2	101.6	2.7	2.24	0.24	0.07	0.01	504.9	1.2	2.14	0.02
9	0.1		101.9		0.08		0.00		503.2		0.08	0.001
10	0.4	0	102.0	0.6	0.38	0.04			**505.7**	**2.9**	0.37	0.004
11	10	1.7	103.0	1.0	10.68	1.71	0.04	0.04	**500.7**	**0.0**	####	0.10
12	11	1.6	101.6	0.2	11.64	1.65	0.18	0.22	**903.1**	**1.1**	####	0.11
13	7.2	0.6	103.4	1.4	7.39	0.49	0.08	0.03	**907.0**	**0.4**	7.08	0.07
14	7.7	4.1	103.2	2.7	7.82	4.07	0.21	0.02	**902.6**	**0.5**	7.50	0.07
16	3.9	0.4	102.1	3.3	4.03	0.36	0.04	0.02	**904.8**	**2.8**	3.91	0.04
17	4.3	0.7	101.8	1.8	4.42	0.73	0.17	0.03	**904.3**	**0.8**	4.17	0.04
18	3.6	0.7	101.6	1.4	3.68	0.75	0.10	0.02	**900.6**	**1.1**	3.51	0.04
19	0.1		100.7		0.07						0.07	0.001
20	0.3	0	101.1	1.9	0.29	0.02	0.01	0.02	**902.4**	**2.6**	0.27	0.003
M4C3	4.7	1.7	98.5	4.3	3.05	2.83	0.03	0.01	**902.6**	**9.8**	4.65	

Appendix B5: Batch reactors- material weights

Label	Rep	Matrix Wt (g)	ZVI (g)	Low ZVI		High ZVI		M1C1		M4C3	
Sand		813.09		6.1	0.036	12.0	0.011	560	11	500	0.1
M4/C3 G0 Fe0	1	499.93									
M4/C3 G0 Fe5	1	500.14	6.1								
M4/C3 G0 Fe10	1	499.94	12.04								
M4/C3 G10 Fe10	1	499.98	12.03								
M4/C3 G30 Fe10	1	500.2	12.04								
M1/C1 G0 Fe0	1	558.02									
M1/C1 G10 Fe10	1	563.65	12.05								
M1/C1 G0 Fe10	1	544.4	12.03								
Sand spk	1	833.24									
Soil spk	1	728.79									
M4/C3 G0 Fe0	2	500.06									
M4/C3 G0 Fe5	2	500.06	6.03								
M4/C3 G0 Fe10	2	500.2	12.01								
M4/C3 G10 Fe10	2	500.03	12.02								
M4/C3 G30 Fe10	2	500.06	12.04								
M1/C1 G0 Fe0	2	567.49									
M1/C1 G10 Fe10	2	545.19	12.02								
M1/C1 G0 Fe10	2	555.73	12.05								
Sand spk	2	809.94									
Soil spk	2	727.77									
M4/C3 G0 Fe0	3	500.15									
M4/C3 G0 Fe5	3	500.16	6.05								
M4/C3 G0 Fe10	3	500.03	12.03								
M4/C3 G10 Fe10	3	500.21	12.03								
M4/C3 G30 Fe10	3	500.16	12.03								
M1/C1 G0 Fe0	3	579.84									
M1/C1 G10 Fe10	3	563.15	12.04								
M1/C1 G0 Fe10	3	558.63	12.04								
Sand spk	3	809.4									
Soil spk	3	732.61									

Appendix B6: Setup for Kd determination

	24 h	Type	M4C3 Wt (g)	Water Vol (mL)	Water Wt (g)	TCE Spike (ug)	RT	gas 1	2	TCE			01-18-15 Std Curve Method TCE (ug/mL)	Corrected from ppmV to ug/mL Method TCE
1	091915001	Crimp	10.063	6.1	6.12	14.0	1.124; 1.167; 1.826	0.15	0.34	1.0	headspace only	0.068	15.3	0.086
2	091915002	Crimp	10.018	6.1	6.11	14.0	1.127; 1.169; 1.828	0.12	1.3	1.1	headspace only	0.074	16.9	0.095
3	091915003	Crimp	10.034	6.1	6.10	14.0	1.124; 1.165; 1.826	0.13	1.2	1.0	headspace only	0.068	15.3	0.086
4	091915004	uQT Vial	10.054	6.1	6.11	14.0	1.169		0.74		headspace only			
5	091915005	uQT Vial	10.100	6.1	6.11	14.0	1.169		0.62		headspace only			
6	091915006	uQT Vial	10.016	6.1	6.10	14.0	1.127; 1.169	0.0092	0.57		headspace only			
7	091915013	Crimp	10.008	full	10.68	14.0	1.126; 1.168; 1.826	0.11	0.28	0.23	600uL liquid left to eq. in GC vial; 600uL HS read	0.016	3.1	0.017
8	091915014	Crimp	10.080	full	10.66	14.0	1.126; 1.169; 1.827	0.11	0.34	0.29	600uL liquid left to eq. in GC vial; 600uL HS read	0.020	4.0	0.023
9	091915015	Crimp	10.005	full	10.88	14.0	1.129; 1.171; 1.828	0.12	0.26	0.30	600uL liquid left to eq. in GC vial; 600uL HS read	0.020	4.2	0.024
10	091915008	uQT Vial	10.138	full	18.01	14.0	1.164; 1.825		1.3	0.27	liquid			
10	091915011	uQT Vial R2	10.138	full	18.01	14.0	1.17		0.48		liquid			
11	091915009	uQT Vial	10.003	full	17.95	14.0	1.127; 1.169; 1.827	0.29	0.47	0.69	liquid			
12	091915010	uQT Vial	10.053	full	18.02	14.0	1.127; 1.169; 1.829	0.097	0.44	0.064	liquid			

w/ HS		SD		H'=	0.181	(12oC)
Avg TCE (ug/mL)=Cg	0.09	0.005	24 h	Cw	0.490	ug/mL
Avg Liq Vol (mL)	6.11	0.01				
Avg Gas Vol (mL)	4.64			Mg	0.411	ug
				Mw	2.99	ug
				Mt	3.406	ug
w/o HS						
Avg TCE (ug/mL)=Cg				Cw		ug/mL
Avg Liq Vol (mL)						
Avg Gas Vol (mL)				Mg		ug
				Mw		ug
				Mt		ug

			SD		H'=	0.181	(12oC)		Ideal		H'=	0.18	(12oC)	Ideal		H'=	0.18	(12oC)
600 uL, headspace	Avg TCE (ug/mL)	0.108	0.03	48h	Cwc	0.59	ug/mL		w/	soil	Cwc	1.13	ug/mL	w/o	soil	Cwc	1.26	ug/mL
600 uL, headspace	Avg TCE (ug/mL)	0.104		72h	Mgc	0.66	ug		Cg	0.20	Mgc	0.95	ug	Cg	0.23	Mgc	1.42	ug
1 uL liquid inj; not mixed	Big Avg TCE (ug/mL) = Cg	0.107	0.02		Mwc	5.87	ug	-->	Vwc	11.58	Mwc	13.1	ug	Vwc	9.98	Mwc	12.58	ug
1 uL liquid inj; mixed	Avg Liquid vol (mL) = Vwc	9.98	0.03		Mt	6.54	ug		Vgc	4.64	Mt	14	ug	Vgc	6.24	Mt	14	ug
1 uL liquid inj; mixed	Avg Gas vol (mL) = Vgc	6.24				???					Check Mt	14.0				Check Mt	14.0	
600 uL, headspace	total volume (mL)	16.22																
600 uL, headspace; R1																		
600 uL, headspace; R2			SD		H'=	0.367	(25oC)		Ms =	(Mgc + Mwc) - (Mg + Mw)				12oC				
1 uL liquid inj; not mixed; 600 uL liquid placed in GC vial for meas tomorrow	Avg TCE (ug/mL) = Cg	0.227	0.04		Cwc	0.62	ug/mL		Ms =	10.59	ug							
1 uL liquid inj; mixed; 600 uL liquid placed in GC vial for meas tomorrow	Avg Liquid vol (mL) = Vwc	9.91	0.06		Mgc	1.43	ug		Cs =	Ms/Ws		Ws =	soil mass	10.04	g			
1 uL liquid inj; mixed; 600 uL liquid placed in GC vial for meas tomorrow	Avg Gas vol (mL) = Vgc	6.31			Mwc	6.11	ug		Cs =	1.06	ug/g = mg/kg							
600uL liquid left to eq. in GC vial; 600uL HS read					Mt	7.54	ug		Kd =	Cs/Cw								
600uL liquid left to eq. in GC vial; 600uL HS read									Kd =	2.15	mL/g = L/kg		M4C3 material					
600uL liquid left to eq. in GC vial; 600uL HS read																		
600 uL, headspace; Room Temp		61.378																
600 uL, headspace; Room Temp		0.2281																

Appendix B7: Determining ZVI density

	Mass (g)	Initial Vol (mL)	Final Vol (mL)	Density	Notes		Approx Vol (mL)	Mass (g)
1	2.13	17.2	17.4	10.65	no settling time	1	2.5	6.28
2	6.72	19.0	20.0	6.72	no settling time	2	2.5	6.42
3	12.01	19.4	21.2	6.67	no settling time	3	2.5	6.28
4	12.05	15.0	16.9	6.34	10 min settle	4	2.5	6.49
5	20.01	16.2	19.2	6.67	10 min settle	5	2.5	6.11
6	20.15	15.2	18.2	6.72	10 min settle	6	2.5	5.33
			18.2	6.72	waited extra 10 min to settle	7	2.5	5.85
	excl. values		*avg ρ =*	*6.62*	*g/mL*	8	2.5	5.57
			stdev =	*0.16*		9	2.5	6.31
						10	2.5	6.23
							avg wt =	*6.09*
							stdev =	*0.38*

Therefore, to achieve ___ vol/L ZVI, use the following:

Want	[--]	mL/L ZVI
density	6.62	g/mL

Use Density * Volume wanted = Mass needed of ZVI to achieve desired volume ZVI

Conc (mL/L)	Vol Needed (mL)	Density	Mass (g)	
5	2.5	6.62	16.55	porosity?
10	5		33.1	
20	10		66.2	

Appendix C1: RDase gene BLAST- *tceA* series

Dehalococcoides mccartyi strain AD14-1 TCE reductive dehalogenase (*tceA*) gene, partial cds

GenBank: **KC342970.1**

LOCUS   KC342970   1589 bp   DNA   linear   BCT 12-JAN-2014
DEFINITION *Dehalococcoides mccartyi* strain AD14-1 TCE reductive
            dehalogenase (*tceA*) gene, partial cds.
VERSION   KC342970.1 GI:545274867
KEYWORDS   .
SOURCE   *Dehalococcoides mccartyi*
  ORGANISM *Dehalococcoides mccartyi*
            Bacteria; Chloroflexi; Dehalococcoidia; Dehalococcoidales;
            Dehalococcoidaceae; Dehalococcoides.
REFERENCE   1 (bases 1 to 1589)
  AUTHORS   Wang,S. and He,J.
  TITLE   Dechlorination of commercial PCBs and other multiple halogenated
            compounds by a sediment-free culture containing *Dehalococcoides* and
            Dehalobacter
  JOURNAL   Environ. Sci. Technol. 47 (18), 10526-10534 (2013)
   PUBMED   23964900
REFERENCE   2 (bases 1 to 1589)
  AUTHORS   Wang,S. and He,J.
  TITLE   Direct Submission
  JOURNAL   Submitted (17-DEC-2012) Department of Civil and Environmental
            Engineering, National University of Singapore, T-Lab Building, #07-
            03; 5A Engineering Drive 1, Singapore 117411, Singapore

TCTACAGTCACAAGGCGTGATTTCATGA**AGAGATTAGGTTTGGCAGGA**GCCGG
TGCGGGGGCACTGGGTGCCGCAGTACTTGCAGAGAATAACCTGCCGCATGAGTT
TAAAGATGTTGATGACCTGCTGTCAGCAGGTAAAGCTTTAGAGGGTGACCACGCT
AATAAAGTAAACAATCATCCATGGTGGGTTACCACGCGTGATCATGAGGATCCA
ACCTGTAATATAGATTGGAGCCTTATAAAAAGATACAGCGGTTGGAACAACCAG
GGAGCATACTTCTTACCTGAGGATTACCTGTCTCCAACCTATACAGGTAGAAGAC
ATACTATTGTTGATTCATCTCTAGAAGTAAAATTACAGGGTAAAAAATACCGTGA
TAGTGCCTTTATAAAATCAGGCATAGACTGGATGAAGGAAAATATTG**ATCCAGA**
TTATGACCCTGGTGAAC**TGGGCTATGGCGACCGCAGG**G**AAGATGCCCTAAT**
ATATGCCGCCACGAATGGCTCACATAATTGCTGGGAGAACCCGCTTTATGGACG
CTATGAAGGTTCTAGGCCTTATCTCTCTATGCGAACCATGAATGGAATAAACGGC
TTGCATGAATTTGGTCACGCAGATATCAAAACCACCAACTACCCGAA**GTGGGAG**
GGTACGCCTGAAGAGAACCTGTTAATCATGCGCACCGCCGCGCGCTACTTCGGG
GCTTCTTCCGTTGGCGCCATTAAGATAACGGATAACGTGAAGAAAATCTTCTATG
CCAAAGCCCAGCCCTTTTGCCTCGGGCCTTGGTATACGATTACAAATATGGCTGA
ATACATTGAATATCCGGTCCCAGTAGATAATTATGCTATACCCATTGTGTTTGAA
GATATCCCTGCAGACGAGGGGCATTACAGCTACAAACGCTTTGGCGGTGATGAT
AAGATAGCAGTACCCAATGCACTGGATAACATCTTCACCTATACCATCATGCTCC

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TGCCTATCCCCTCTTTACAGAGGTTGAGGCACGCATTCAGCAATTCATTGCAGGC
CTTGGCTATAACTCGATGGGTGGTGGAGTTGAAGCTTGGGGTCCGGGCAGTGCCT
TCGGCAACTTAAGTGGCCTTGGGGAACAATCACGCGTATCAAGCACTATTGAGCC
CCGCTACGGTTC**CAACACCAAGGGTTCCCTA**AGGATGCTTACCGACCTGCCTCT
TGCCCCCACCAAGCCTATAGATGCCGGTATCCGTGAGTTCTGTAAGACCTGCGGC
ATCTGTGCCGAGCATTGTCCTACCCAAGCTATCTCGCATGAAGGGCCGCGCTATG
ACTCACCACACTGGGATTGCGTAAGCGGTTATGAGGGCTGGCACCTTGACTATCA
CAAATGCACTAACTGTACCATCTGTGAGGCCG**TCTGCCCCTTCTTCACTATG**AG
CAATAACTCCTGGGTGCACAACTTGGTCAAGTCCACTGTTGCCACTACGCCCGTT
TTTAACGGTTTCTTTAAGAATATGGAAGGAGCCTTCGGCTACGGCCCGCGCTACT
CACCAAGCAG

Deth_tceAF 5’- **AGAGATTAGGTTTGGCAGGA**
Deth_tceAR 5’- CATAGTGAAGAAGGGGCAGA
Deth_tceAR 3’- **TCTGCCCCTTCTTCACTATG** (reverse complement)
Deth_tceAF/R expected size 1439bp

Deth_tceAD2F 5’- **GTGGGAGGGTACGCCTGAAG**
Deth_tceAD4R 5’- TAGGGAACCCTTGGTGTTG
Deth_tceAD4R 3’- **CAACACCAAGGGTTCCCTA** (reverse complement)
Deth_tceAD2F/4R expected size 586bp

797F 5’- ACGCCAAAGTGCGAAAAGC
2490R 5’- TAATCTATTCCATCCTTTCTC
2490R 3’- GAGAAAGGATGGAATAGATTA (reverse complement)
797F/2490R expected size 1732bp (sequence is outside fragment)
TceA1270F 5’- **ATCCAGATTATGACCCTGGTGAA**-3’
TceA1336R 5’- GCGGCATATATTAGGGCATCTT-3’
TceA1336R 3’- **AAGATGCCCTAATATATGCCGC**-5’ (reverse complement)
TceAF/R expected size 67bp

TceA1294 5’-FAM-**TGGGCTATGGCGACCGCAGG**-TAMRA-3’

Appendix C2: RDase gene BLAST- *vcrA* series

Uncultured *Dehalococcoides* sp. *vcrA, vcrB* genes for vinyl chloride reductase, putative membrane anchor for vinyl chloride reductase, complete cds, clone: F-v2

GenBank: **AB586010.1**

LOCUS AB586010 2191 bp DNA linear ENV 15-NOV-2011
DEFINITION Uncultured *Dehalococcoides* sp. *vcrA, vcrB* genes for vinyl chloride reductase, putative membrane anchor for vinnyl chloride reductase, complete cds, clone: F-v2.
ACCESSION AB586010
VERSION AB586010.1 GI:356995590
KEYWORDS ENV.
SOURCE uncultured *Dehalococcoides* sp.
ORGANISM uncultured *Dehalococcoides* sp.
Bacteria; Chloroflexi; Dehalococcoidia; Dehalococcoidales; Dehalococcoidaceae; Dehalococcoides; environmental samples.
REFERENCE 1
AUTHORS Nakamura,K., Ara,S., Mizumoto,M., Ueno,T. and Ishida,H.
TITLE Cloning and analysis of vinyl chloride reductase genes and their detection at cleanup sites
JOURNAL Environ. Eng. Res. 47 (2010) In press
REFERENCE 2 (bases 1 to 2191)
AUTHORS Nakamura,K., Mizumoto,M., Ueno,T. and Ishida,H.
TITLE Direct Submission
JOURNAL Submitted (02-SEP-2010) Contact:Kanji Nakamura Tohoku Gakuin University, Department of Civil and Environmental Engineering; 1-13-1, Chuo, Tagajyo-shi, Miyagi-ken 985-8537, Japan

TGATGGGGTTTGCAGGATGTTAAGATGCAAAAATAACTGTAGTTTTTATTATTTAAAGTA
ACTCGTGTTCGATATGTAGTAAATTAGCAAAGACTAGTACTTAAGTATTAATACTTAAGTA
CATTTATTTTATATATCTTTGCGTATTTTGTGCAATTGACATTACTATATAAAATGCTAGAA
TACAAATAATCAACTGTGTATTTGTACTGAGGAAACGCTTATGGATATTTGGCGTTCAGGA
AAGACTGAATGGCTCTAGGGAAAGACCTAAATATATCTTTAGGATAAATTTATGAGTAAA
TTTCATAAAACGATTAGCCGCCGAGATTTCATGAAAGGACTAGGATTAGCCGGGGCAGGC
ATAGGCGCTGTTGCGGCGTCAGCTCCGGTTTTTCATGACATTGATGAATTTGTTTCAAGCG
AAGCAAATTCTACTAAAGATCAACCTTGGTACGTTAAGCATCGAGAGCATTTTGACCCTA
CGATTACAGTTGACTGGGATATTTTTGATAGATATGACGGGTATCAGCATAAGGGTGTCT
ATGAAGGCCCTCCAGATGCTCCCTTTACATCATGGGGCAATAGGCTTCAGATGAGAATGT
CAGGTGAAGAGCAAAAGAAGCGAATTTTGGCCGCTAAAAAAGAGAGGTTCCCTGGTTGG
GACGGTGGGTTACACGGGAGAGGGGATCAGCGGGCGGATGCACTATTTTACGCAGTAACT
CAACCATTTCCTGGTAGTGGTGAGGAAGGGCACGGACTATTCCAACCTTATCCTGATCAA
CCCGGTAAGTTTTACGCGAGATGGGGTTTGTATGGTCCGCCACATGATTCAGCGCCACCTG
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CCTATTAACTTTAAGGAAGCGGATTATAGCTACTACAATGATGCAGAGTGGGTTATTCCA
ACAAAGTGTGAATCCATTTTCACTTTCACCCTACCTCAACCACAAGAACTCAATAAGAGG
ACGGGTGGTATAGCAGGTGCTGGATCATATACTGTATACAAAGATTTCGCTAGGGTAGGC
ACTTTAGTCCAATTGTTTATTAAGAATCTAGGTTATCACGCTTTATATTGGCCAATTGGAT
GGGGACCGGGTGGTTGCTTTACCACTTTTGACGGCAAGGTGAACAGGGTAGAACAGGTG
CTGCTATCCATTGGAAGTTTGGTTCTTCACAACGTGGTTCTGAAAGAGTAGTAACTGATTT

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ATATGCCGTGACGTTTGCGTCTCTGGGGGTGTGCACCAAGAAGACGAACCAACTTGGGAT
TCAGGTAATTGGTGGAATGTACAAGGATAT**CTCGGCTACCGAACGGATT**G**GAGTGGTT**
GCCATAACCAGTGCGGT**ATGTGTCAATCCTCCTGCCC**TTTTACTTATTTAGGTTTGGAA
AATGCTTCATTAGTGCACAAAATAGTAAAAGGTGTTGTTGCTAACACGACTGTTTTTAATA
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CATTAGCAGTTGGACTAACTATGCTATTTACCTGGTTTAAAAAGAATAATATCACTTTAAA
GTGGAATGAGTGGGTACTTGGCATATTGGGGCTGTTACTAGCTTTGTTTGCTATTCAACAC
ACATATGCCAGTGCTACATATGAATTTGAATATACGTCAGCATGGATAGTGGGCGTCATA
GTGTTATTGTTAGCTGTAGTACCGTTGTTATTTGCGGCAAGATCAGTAAGACGCAGGGTAG
ACAAATAACGGGTATCTTTAAG

vcrAB_F 5'- **CTATGAAGGCCCTCCAGATGC** (DOES NOT EXIST HERE)
vcrAB_R 5'- GTAACAGCCCCAATATGCAAGT
vcrAB_R 3'- **ACTTGCATATTGGGGCTGTTAC** (reverse complement)
F/R expected size 1482bp

vcrA_F 5'-**CTCGGCTACCGAACGGATT**-3'
vcrA_R 5'-GGGCAGGAGGATTGACACAT-3'
vcrA_R 3'-**ATGTGTCAATCCTCCTGCCC**-5' (reverse complement)
F/R expected size: 65bp

vcrA_Probe 5'-FAM-CGCACTGGTTATGGCAACCACTC-TAMRA-3'
vcrA_Probe 3'-**GAGTGGTTGCCATAACCAGTGCG**-5' (reverse complement)

Dehalococcoides sp. ANAS2 reductive dehalogenase _VcrA_ (_vcrA_) gene, complete cds

GenBank: HM241732.2

LOCUS HM241732 3782 bp DNA linear BCT 06-FEB-2012
DEFINITION *Dehalococcoides* sp. ANAS2 reductive dehalogenase *VcrA (vcrA)* gene, complete cds.
ACCESSION HM241732
VERSION HM241732.2 GI:374713104
KEYWORDS .
SOURCE *Dehalococcoides* sp. ANAS2
ORGANISM *Dehalococcoides* sp. ANAS2
Bacteria; Chloroflexi; Dehalococcoidia; Dehalococcoidales; Dehalococcoidaceae; Dehalococcoides.
REFERENCE 1 (bases 1 to 3782)
AUTHORS Lee,P.K., Cheng,D., Hu,P., West,K.A., Dick,G.J., Brodie,E.L., Andersen,G.L., Zinder,S.H., He,J. and Alvarez-Cohen,L.
TITLE Comparative genomics of two newly isolated Dehalococcoides strains and an enrichment using a genus microarray
JOURNAL ISME J (2011) In press
PUBMED 21228894
REMARK Publication Status: Available-Online prior to print
REFERENCE 2 (bases 1 to 3782)
AUTHORS Lee,P.K.H., Cheng,D., Hu,P., West,K.A., Dick,G.J., Brodie,E.L.,

Andersen,G.L., Zinder,S.H., He,J. and Alvarez-Cohen,L.
TITLE Direct Submission
JOURNAL Submitted (19-MAY-2010) Environmental Science and Engineering, National University of Singapore, T-Lab, 5A Engineering Drive 1, Singapore 117411, Singapore
REFERENCE 3 (bases 1 to 3782)
AUTHORS Cheng,D., Wu,Y., Lee,P.K.H., Alvarez-Cohen,L. and He,J.
TITLE Direct Submission
JOURNAL Submitted (31-JAN-2011) Civil and Environmental Engineering, National University of Singapore, 5A Engineering Drive 1, Singapore 117411, Singapore

CTTTGGCTATGAATCCTGTTGGGAGATAGGCGACAAAGGAGAAAAGAAAAGGCGCTGCG
AGCCGGAACACCCCGGAGGACTTCATATCCACTCAAGGGAAGGGCCAGCAGCAGCCGAG
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CGGGCAGATAAACCACAGGGATGAAATCCTAGCCGGTGCCCATGAGGCCTTAATCAGCCG
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GTTCAGGAAAGACTGAATGGCTCTAGGGAAAGACCTAAATATATCTTTAGGATAAATTTA
TGAGTAAATTTCATAAAACGATTAGCCGCCGAGATTTCATGAAAGGACTAGGATTAGCCG
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TTCAAGCGAAGCAAATTCTACTAAAGATCAACCTTGGTACGTTAAGCATCGAGAGCATTT
TGACCCTACGATTACAGTTGACTGGGATATTTTTGATAGATATAACGGGTATCAGCATAA
GGGTGT**CTATGAAGGCCCTCCAGATGC**TCCCTTTACATCATGGGGCAATAGGCTTCAGA
CGAGAATGTCAGATGAAGAGCAAAAGAAGCGAATTTTGGCCGCTAAAAAAGAGAGGTTC
CCTGGTTGGGACGGTGGGTTACACGGGAGAGGGGATCAGCGGGCGGATGCACTATTTTAC
GCAGTAACTCAACCATTTCCTGGTAGTGGTGAGGAAGGGCACGGACTATTCCAACCTTAT
CCTGATCAACCCGGTAAGTTTTACGCGAGATGGGGTTTGTATGGTCCGCCACATGATTCAG
CGCCACCTGATGGGAGCGTACCAAAATGGGAGGGTACTCCAGAAGACAATTTTCTAATGC
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CAAATGCAAAAAACTAATATATAAGAAAGCTCAGCCGATGACTCTAGGAAAAGGAACAT
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GGTAGGCACTTTAGTCCAAATGTTTATTAAGAATCTAGGTTATCACGCTTTATATTGGCCA
ATTGGATGGGGACCGGGTGGTTGCTTTACCACTTTTGACGGGCAAGGTGAACAGGGTAGA
ACAGGTGCTGCTATCCATTGGAAGCTTGGTTCTTCACAACGTGGTTCTGAAAGAGTAGTA
ACTGATTTACCGATAGCTCCTACCCCGCCAATTGATGCAGGTATGTTTAAGTTTTGCAAAA
CCTGTTATATATGCCGTGACGTTTGCGTCTCTGGGGGTGTGCACCAAGAAGACGAACCAA

CTTGGGATTCAGGTAATTGGTGGAATGTACAAGGATAT**CTCGGCTACCGAACGGATT**G**G**
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TTTAATAGTTTTTTTACCAATATGGAGAAAGCATTAGGATATGGTGATTTAACCATGGAAA
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ACAATTGCATTAGCAGTTGGACTAACTATGCTATTTACCTGGTTTAAAAAGAATAATATCA
CTTTAAAGTGGAATGAGTGGGTACTTGGCATATTGGGGCTGTTACTAGCTTTGTTTGCTAT
TCAACACACATATGCCAGTGCTACATATGAATTTGAATATACGTCAGCATGGATAGTGGG
CGTCATAGTGTTATTGTTAGCTGTAGTACCGTTGTTATTTGCGGCAAGATCAGTAAGACGC
AGGGTAGACAAATAACGGGTATCTTTAAGTAGATAAGGTAGTAAGTCGCTTGGGTTGCAC
GCTTAATAAAGCAATCAAGAGCTATTTCATAAAAGTTAACCCCCGAGTTGTATTACCCGT
GGCAACTCGGGGGATTATAGTTTTCTTTTATGTGCAAATATAACGAGAAAATGGATACAA
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TATGGAAATACCGGCGGCGATCAAGATATTGATGCGTATCTAGCTGAAGCTTTACCTGAA
GCGCAAGAGTTTTCTCTTTTGCTTGAAGGTTCGAACGGGGACAACTATTTATATGGCGGAT
ATGATGCTAATATATTAGGTTATGTTGCTGTATCAGAAGGCTATGGTTATGGTGGCCCTCT
TACGGTTGTTACAACCTATAGTACAGATGGCACAATCGCAGCAATAACCGTT

vcrAB_F 5'- **CTATGAAGGCCCTCCAGATGC**
vcrAB_R 5'- GTAACAGCCCCAATATGCAAGT
vcrAB_R 3'- **ACTTGCATATTGGGGCTGTTAC** (reverse complement)
F/R expected size 1482bp

vcrA_F 5'-**CTCGGCTACCGAACGGATT**-3'
vcrA_R 5'-GGGCAGGAGGATTGACACAT-3'
vcrA_R 3'-**ATGTGTCAATCCTCCTGCCC**-5' (reverse complement)

F/R expected size: 65 bp

vcrA_Probe 5'-FAM-CGCACTGGTTATGGCAACCACTC-TAMRA-3'
vcrA_Probe 3'-**GAGTGGTTGCCATAACCAGTGCG**-5' (reverse complement)

Appendix C3: RDase gene BLAST- *bvcA* series

Uncultured *Dehalococcoides* sp. *bvcA, bvcB* genes for vinyl chloride reductase, putative membrane anchor for vinnyl chloride reductase, complete cds, clone: F-b2

GenBank: AB586019.1

LOCUS AB586019 2069 bp DNA linear ENV 15-NOV-2011
DEFINITION Uncultured Dehalococcoides sp. bvcA, bvcB genes for vinyl chloride reductase, putative membrane anchor for vinnyl chloride reductase, complete cds, clone: F-b2.
ACCESSION AB586019
VERSION AB586019.1 GI:356995617
KEYWORDS ENV.
SOURCE uncultured *Dehalococcoides* sp.
ORGANISM uncultured *Dehalococcoides* sp.
Bacteria; Chloroflexi; Dehalococcoidia; Dehalococcoidales; Dehalococcoidaceae; Dehalococcoides; environmental samples.
REFERENCE 1
AUTHORS Nakamura,K., Ara,S., Mizumoto,M., Ueno,T. and Ishida,H.
TITLE Cloning and analysis of vinyl chloride reductase genes and their detection at cleanup sites
JOURNAL Environ. Eng. Res. 47 (2010) In press
REFERENCE 2 (bases 1 to 2069)
AUTHORS Nakamura,K., Mizumoto,M., Ueno,T. and Ishida,H.
TITLE Direct Submission
JOURNAL Submitted (02-SEP-2010) Contact:Kanji Nakamura Tohoku Gakuin, University, Department of Civil and Environmental Engineering; 1-13-1, Chuo, Tagajyo-shi, Miyagi-ken 985-8537, Japan

1-2069

gaccggaaaatcgccaaatatgaattaaaaaagaggggaatatgcataatttccattgtacgataagtaggcgagattttatgaagggat
tggggttagcgggagcagggata**ggtgccgcgacttcagtt**atgccgaattttcacgacttggatgaagt**aatttctgctgctagtgc**
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agagggactttaaagtccctctatgtattattacatttgtacagagtaccttga

bvcA F 5'-**TGCCTCAAGTACAGGTGGT**
bvcA R 5'-ATTGTGGAGGACCTACCT
bvcA R 3’-**AGGTAGGTCCTCCACAAT** (reverse complement)
F/R estimated size 839bp

bvcA_FOR 5'-**GGTGCCGCGACTTCAGTT**
bvcA_REV 5'-TCGGCACTAGCAGCAGAAATT
bvcA_REV 3’-**AATTTCTGCTGCTAGTGCCGA** (reverse complement)
F/R estimated size 68bp

Appendix C4: *Dehalococcoides* spp. Primer BLAST

Dehalococcoides sp. enrichment culture clone BDNP5T-1 16S ribosomal RNA gene, partial sequence

GenBank: **KF305119.1**

LOCUS KF305119 450 bp DNA linear ENV 05-AUG-2013
DEFINITION *Dehalococcoides* sp. enrichment culture clone BDNP5T-1 16S ribosomal RNA gene, partial sequence.
ACCESSION KF305119
VERSION KF305119.1 GI:528081165
KEYWORDS ENV.
SOURCE *Dehalococcoides s*p. enrichment culture clone BDNP5T-1
ORGANISM *Dehalococcoides* sp. enrichment culture clone BDNP5T-1
Bacteria; Chloroflexi; Dehalococcoidia; Dehalococcoidales; Dehalococcoidaceae; Dehalococcoides; environmental samples.
REFERENCE 1 (bases 1 to 450)
AUTHORS Dang,H.T.C., Dinh,H.T.T. and Nguyen,T.T.T.
TITLE Direct Submission
JOURNAL Submitted (29-JUN-2013) EBR Lab, IBT, 18 Hoang Quoc Viet, Cau Giay, Ha Noi 0844, Vietnam

TAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGGAGCGTGTG
GTTTAATTCGATGCTACACGAAGAACCTTACCAAGATTTGACATGCATGA
AGTAGTGAACCGAAAGGGAAACGACCTGTTAAGTCAGGAGTTTGCACAG
GTGCTGCATGGCTGTCGTCAGCTCGTGCCGTGAGGTGTTTGGTTAAGTCCT
GCAACGAGCGCAACCCTTGTTGCTAGTTAAATTTTCTAGCGAGACTGCCC
CGCGAAACGGGGAGGAAGGTGGGGATGACGTCAAGTCAGCATGGCCTTT
ATATCTTGGGCTACACACACGCTACAATGGACAGAACAATAGGTTGCAAC
AGTGTGAA**CTGGAGCTAATCCCCAAAGCT**G**TCCTCAGTTCGGATTGCA**
GGCTGAAA**CCCGCCTGCATGAAGTTGGAGTTG**CTAGTAACCGCATATC
AGCAAG

Dhc1200F 5’-**CTGGAGCTAATCCCCAAAGCT**-3’
Dhc1271R 5’- CAACTTCATGCAGGCGGG-3’
Dhc1271R 3’- **CCCGCCTGCATGAAGTTG**-5’ (reverse complement)
Dhc1200F/R expected size 72bp
Dhc1240
5_-FAM-**TCCTCAGTTCGGATTGCAGGCTGAA**-TAMRA

Appendix D1: Illumina data, family taxonomy of each sample with 0.1%, 0.5%, 1.0% exclusion explored.

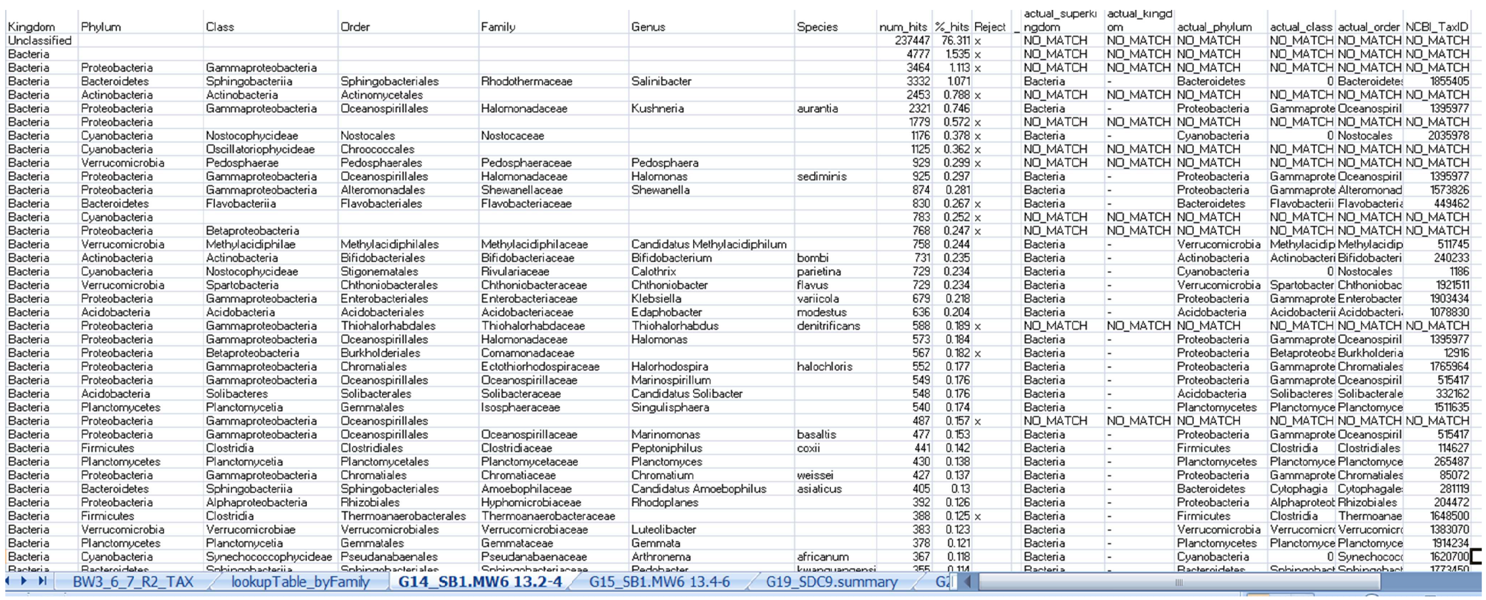
Ref ?	Family member	BV3 deep	Incl. BV3dp (>0.05%	Incl. BV3dp (>0.1%)	Incl. BV3dp (>1.0%)	BV3 shallow	Incl. BV3sh (>0.05%	Incl. BV3sh (>0.1%)	Incl. BV3sh (>1.0%)	BV6 deep	Incl. BV6dp (>0.05%	Incl. BV6dp (>0.1%)	Incl. BV6dp (>1.0%)	BV6 shallow	Incl. BV6sh (>0.05%	Incl. BV6sh (>0.1%)	Incl. BV6sh (>1.0%)	BV8	Incl. BV8 (>0.05%)	Incl. BV8 (>0.1%	Incl. BV8 (>1.0%	MV6 13.2-4	Incl. MV6 2-4 (>0.05%)	Incl. MV6 2-4 (>0.1%)	Incl. MV6 2-4 (1.0%)
	Acetobacteraceae	76	x	x	o	91	x	x	o	296	x	x	o	123	x	x	o	26	x	x	o	426	x	x	o
	Acholeplasmataceae	112	x	x	o	115	x	x	o	371	x	x	o	191	x	x	o	66	x	x	o	27	x	BL	o
	Acidimicrobiaceae	24	x	x	o	51	x	x	o	111	x	x	o	51	x	x	o	22	x	x	o	31	x	BL	o
	Acidithiobacillaceae		N	N	N		N	N	N		N	N	N		N	N	N		N	N	N	3	BL	BL	o
	Acidobacteriaceae	96	x	x	o	62	x	x	o	698	x	x	x	290	x	x	o	64	x	x	o	696	x	x	x
	Actinomycetaceae	22	x	x	o	34	x	BL	o	61	x	BL	o	24	x	BL	o	9	x	BL	o	9	BL	BL	o
	Actinopolysporaceae	21	x	x	o	36	x	BL	o	70	x	x	o	42	x	x	o	23	x	x	o		N	N	N
	Aerococcaceae	10	BL	BL	o	3	BL	BL	o	14	BL	BL	o	2	BL	BL	o		N	N	N	5	BL	BL	o
	Aeromonadaceae	2	BL	BL	o		N	N	N		N	N	N		N	N	N		N	N	N	148	x	x	o
	Alcaligenaceae	14	x	BL	o	24	x	BL	o	29	BL	BL	o	12	BL	BL	o	9	x	BL	o	35	x	BL	o
	Alcanivoracaceae		N	N	N	1	BL	BL	o	2	BL	BL	o		N	N	N		N	N	N	216	x	x	o
	Alicyclobacillaceae	1	BL	BL	o	455	x	x	x	6	BL	BL	o	4	BL	BL	o	3	BL	BL	o		N	N	N
	Alteromonadaceae	19	x	BL	o	12	BL	BL	o	29	BL	BL	o	11	BL	BL	o	8	x	BL	o	579	x	x	x
	Amoebophilaceae	7	BL	BL	o	34	x	BL	o	38	x	BL	o	25	x	BL	o	8	x	BL	o	405	x	x	o
	Anaeroplasmataceae	3	BL	BL	o	1	BL	BL	o	41	x	BL	o	11	BL	BL	o	2	BL	BL	o	1	BL	BL	o
	Anaplasmataceae	14	x	BL	o	33	x	BL	o	95	x	x	o	25	x	BL	o	11	x	BL	o	9	BL	BL	o
	Armatimonadaceae	13	x	BL	o	42	x	x	o	61	x	BL	o	42	x	x	o	8	x	BL	o	1	BL	BL	o
	Aurantimonadaceae	8	BL	BL	o	4	BL	BL	o	13	BL	BL	o	11	BL	BL	o	3	BL	BL	o	1	BL	BL	o
y	Bacillaceae	646	x	x	x	10344	x	x	x	1224	x	x	x	1579	x	x	x	378	x	x	x	798	x	x	x
	Bacteriovoracaceae		N	N	N		N	N	N	1	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Bacteroidaceae	23	x	x	o	19	BL	BL	o	503	x	x	o	130	x	x	o	10	x	BL	o	51	x	x	o
	Balneolaceae		N	N	N	2	BL	BL	o		N	N	N		N	N	N		N	N	N	7	BL	BL	o
	Bartonellaceae	17	x	BL	o	35	x	BL	o	52	x	BL	o	22	x	BL	o	27	x	x	o	3	BL	BL	o
	Bdellovibrionaceae	11	x	BL	o	26	x	BL	o	20	BL	BL	o	7	BL	BL	o	12	x	BL	o	1	BL	BL	o
	Beijerinckiaceae	34	x	x	o	15	BL	BL	o	183	x	x	o	58	x	x	o	17	x	x	o	8	BL	BL	o
	Bifidobacteriaceae	181	x	x	o	341	x	x	x	745	x	x	x	360	x	x	x	152	x	x	x	733	x	x	x
	Bogoriellaceae	4	BL	BL	o	1	BL	BL	o	7	BL	BL	o	3	BL	BL	o		N	N	N		N	N	N
	Borreliaceae	12	x	BL	o	18	BL	BL	o	32	BL	BL	o	5	BL	BL	o	9	x	BL	o	25	x	BL	o
	Brachyspiraceae	11	x	BL	o	19	BL	BL	o	24	BL	BL	o	12	BL	BL	o	7	x	BL	o	1	BL	BL	o
	Bradyrhizobiaceae	94	x	x	o	129	x	x	o	660	x	x	o	246	x	x	o	71	x	x	o	51	x	x	o
	Brevibacteriaceae	1	BL	BL	o	1	BL	BL	o	2	BL	BL	o	3	BL	BL	o		N	N	N	2	BL	BL	o
	Brucellaceae	3	BL	BL	o	5	BL	BL	o	4	BL	BL	o	6	BL	BL	o	3	BL	BL	o	5	BL	BL	o
y	Burkholderiaceae	105	x	x	o	183	x	x	o	264	x	x	o	80	x	x	o	66	x	x	o	298	x	x	o
	Caldilineaceae	325	x	x	x	417	x	x	x	1145	x	x	x	514	x	x	x	204	x	x	x	54	x	x	o
y	Campylobacteraceae	11	x	BL	o	27	x	BL	o	103	x	x	o	39	x	x	o	7	x	BL	o	11	BL	BL	o
y	Carnobacteriaceae	466	x	x	x	55	x	x	o	1417	x	x	x	517	x	x	x	197	x	x	x	3	BL	BL	o
	Catenulisporaceae		N	N	N		N	N	N	3	BL	BL	o	1	BL	BL	o		N	N	N		N	N	N
	Caulobacteraceae	79	x	x	o	82	x	x	o	114	x	x	o	86	x	x	o	54	x	x	o	109	x	x	o
	Cellulomonadaceae	45	x	x	o	54	x	x	o	415	x	x	o	134	x	x	o	18	x	x	o	154	x	x	o
	Chitinophagaceae	334	x	x	x	511	x	x	x	817	x	x	x	233	x	x	o	196	x	x	x	319	x	x	o
	Chlorobiaceae	14	x	BL	o	29	x	BL	o	82	x	x	o	24	x	BL	o	17	x	x	o	1	BL	BL	o
	Chromatiaceae	82	x	x	o	130	x	x	o	256	x	x	o	98	x	x	o	40	x	x	o	928	x	x	x
	Chroococcaceae	5	BL	BL	o	7	BL	BL	o	8	BL	BL	o	2	BL	BL	o		N	N	N	21	BL	BL	o
	Chrysiogenaceae	2	BL	BL	o	1	BL	BL	o	16	BL	BL	o	1	BL	BL	o	1	BL	BL	o		N	N	N
	Chthoniobacteraceae	44	x	x	o	99	x	x	o	662	x	x	o	304	x	x	o	74	x	x	o	782	x	x	x
	Chthonomonadaceae	4	BL	BL	o	4	BL	BL	o	33	BL	BL	o	29	x	BL	o		N	N	N	128	x	x	o
	Chthonomonadaceae	4	BL	BL	o	4	BL	BL	o	33	BL	BL	o	29	x	BL	o		N	N	N	128	x	x	o
y	Clostridiaceae	3166	x	x	x	3503	x	x	x	12228	x	x	x	5983	x	x	x	2128	x	x	x	1175	x	x	x
	Comamonadaceae	197	x	x	o	294	x	x	o	233	x	x	o	93	x	x	o	110	x	x	o	1639	x	x	x
	Conexibacteraceae	24	x	x	o	41	x	x	o	91	x	x	o	42	x	x	o	21	x	x	o		N	N	N
	Coriobacteriaceae	75	x	x	o	56	x	x	o	237	x	x	o	114	x	x	o	26	x	x	o	16	BL	BL	o
	Corynebacteriaceae	1	BL	BL	o	4	BL	BL	o	8	BL	BL	o	3	BL	BL	o	1	BL	BL	o	4	BL	BL	o
	Coxiellaceae	74	x	x	o	110	x	x	o	292	x	x	o	141	x	x	o	28	x	x	o	29	x	BL	o
	Crenotrichaceae	8	BL	BL	o	1	BL	BL	o	25	BL	BL	o	17	x	BL	o	1	BL	BL	o		N	N	N
	Cryomorphaceae	3	BL	BL	o	2	BL	BL	o	2	BL	BL	o		N	N	N	2	BL	BL	o	61	x	x	o
	Cyanobacteriaceae		N	N	N		N	N	N	2	BL	BL	o		N	N	N	1	BL	BL	o	5	BL	BL	o
	Deferribacteraceae	15	x	BL	o	41	x	x	o	72	x	x	o	23	x	BL	o	16	x	x	o	7	BL	BL	o
y	Dehalococcoidaceae	5	BL	BL	o	13	BL	BL	o	31	BL	BL	o	11	BL	BL	o	6	BL	BL	o		N	N	N
	Deinococcaceae	1183	x	x	x	1199	x	x	x	4102	x	x	x	1525	x	x	x	634	x	x	x	83	x	x	o
	Dermabacteraceae	6	BL	BL	o	10	BL	BL	o	10	BL	BL	o	5	BL	BL	o	1	BL	BL	o	1	BL	BL	o
	Dermacoccaceae		N	N	N		N	N	N	1	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Desulfobacteraceae	246	x	x	x	377	x	x	o	490	x	x	o	221	x	x	o	99	x	x	o	137	x	x	o
	Desulfobulbaceae	17	x	BL	o	4	BL	BL	o	16	BL	BL	o	13	BL	BL	o	9	x	BL	o	125	x	x	o
	Desulfohalobiaceae	120	x	x	o	86	x	x	o	269	x	x	o	130	x	x	o	49	x	x	o	4	BL	BL	o
	Desulfomicrobiaceae	7	BL	BL	o	1	BL	BL	o	4	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Desulfovibrionaceae	202	x	x	o	306	x	x	o	576	x	x	o	257	x	x	o	167	x	x	x	202	x	x	o
	Desulfurococcaceae		N	N	N	2	BL	BL	o	2	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Desulfuromonadaceae	26	x	x	o	26	x	BL	o	106	x	x	o	44	x	x	o	22	x	x	o	259	x	x	o
	Dietziaceae		N	N	N	2	BL	BL	o		N	N	N		N	N	N	1	BL	BL	o	2	BL	BL	o
	Ectothiorhodospiraceae	116	x	x	o	154	x	x	o	270	x	x	o	150	x	x	o	73	x	x	o	904	x	x	x
y	Enterobacteriaceae	4	BL	BL	o	8	BL	BL	o	29	BL	BL	o	10	BL	BL	o	4	BL	BL	o	1097	x	x	x
	Enterococcaceae	58	x	x	o	72	x	x	o	253	x	x	o	116	x	x	o	62	x	x	o	24	x	BL	o
	Entomoplasmataceae	1	BL	BL	o		N	N	N	10	BL	BL	o	4	BL	BL	o	3	BL	BL	o	2	BL	BL	o
	Erysipelotrichaceae	224	x	x	x	58	x	x	o	946	x	x	x	308	x	x	x	33	x	x	o		N	N	N
	Erythrobacteraceae	54	x	x	o	89	x	x	o	109	x	x	o	55	x	x	o	32	x	x	o	101	x	x	o
y	Eubacteriaceae	3	BL	BL	o	12	BL	BL	o	20	BL	BL	o	8	BL	BL	o	3	BL	BL	o	5	BL	BL	o
	Euzebyaceae	10	BL	BL	o	18	BL	BL	o	47	x	BL	o	21	x	BL	o	17	x	x	o	54	x	x	o
	Ferrimonadaceae		N	N	N	3	BL	BL	o	1	BL	BL	o	6	BL	BL	o		N	N	N		N	N	N
	Fibrobacteraceae	1	BL	BL	o		N	N	N	4	BL	BL	o	2	BL	BL	o		N	N	N		N	N	N
	Fimbriimonadaceae	5	BL	BL	o	8	BL	BL	o	17	BL	BL	o		N	N	N	2	BL	BL	o		N	N	N
	Flammeovirgaceae	7	BL	BL	o	8	BL	BL	o	15	BL	BL	o	6	BL	BL	o	4	BL	BL	o	2	BL	BL	o
y	Flavobacteriaceae	714	x	x	x	707	x	x	x	2804	x	x	x	1295	x	x	x	417	x	x	x	577	x	x	x
	Francisellaceae	1	BL	BL	o		N	N	N	4	BL	BL	o		N	N	N	3	BL	BL	o	3	BL	BL	o
	Frankiaceae	4	BL	BL	o		N	N	N	14	BL	BL	o	2	BL	BL	o	3	BL	BL	o		N	N	N
	Fusobacteriaceae		N	N	N		N	N	N	1	BL	BL	o		N	N	N		N	N	N	92	x	x	o
	Gallionellaceae	3	BL	BL	o	4	BL	BL	o	2	BL	BL	o		N	N	N	2	BL	BL	o	118	x	x	o
	Gemmataceae	31	x	x	o	25	x	BL	o	146	x	x	o	54	x	x	o	26	x	x	o	408	x	x	o
	Gemmatimonadaceae	14	x	BL	o	26	x	x	o	93	x	x	o	48	x	x	o	12	x	BL	o	62	x	x	o
y	Geobacteraceae	463	x	x	x	476	x	x	x	1739	x	x	x	671	x	x	x	202	x	x	x	454	x	x	x
	Geodermatophilaceae	2	BL	BL	o	1	BL	BL	o		N	N	N		N	N	N		N	N	N		N	N	N
	Glycomycetaceae	7	BL	BL	o	8	BL	BL	o	18	BL	BL	o	12	BL	BL	o	3	BL	BL	o	3	BL	BL	o
	Gomphosphaeriaceae	4	BL	BL	o	4	BL	BL	o	13	BL	BL	o	3	BL	BL	o	4	BL	BL	o	60	x	x	o
	Gordoniaceae	4	BL	BL	o		N	N	N	7	BL	BL	o		N	N	N	1	BL	BL	o		N	N	N
	Hahellaceae	1	BL	BL	o	3	BL	BL	o		N	N	N	1	BL	BL	o	1	BL	BL	o		N	N	N
	Halanaerobiaceae	3	BL	BL	o	4	BL	BL	o	11	BL	BL	o	3	BL	BL	o	3	BL	BL	o		N	N	N
	Halobacteriaceae	3	BL	BL	o	3	BL	BL	o		N	N	N	2	BL	BL	o		N	N	N	100	x	x	o
	Halobacteroidaceae		N	N	N	5	BL	BL	o		N	N	N		N	N	N	2	BL	BL	o	1	BL	BL	o
	Halomonadaceae	37	x	x	o	41	x	x	o	60	x	BL	o	21	x	BL	o	30	x	x	o	4181	x	x	x
	Halothiobacillaceae		N	N	N	1	BL	BL	o	1	BL	BL	o		N	N	N		N	N	N	25	x	BL	o
	Helicobacteraceae	23	x	x	o	43	x	x	o	35	x	BL	o	29	x	BL	o	18	x	x	o	5	BL	BL	o
	Heliobacteriaceae	65	x	x	o	114	x	x	o	156	x	x	o	49	x	x	o	28	x	x	o	12	BL	BL	o
	Holophagaceae	77	x	x	o	82	x	x	o	457	x	x	o	150	x	x	o	36	x	x	o	85	x	x	o
	Hydrogenophilaceae	11	x	BL	o	21	x	BL	o	28	BL	BL	o	10	BL	BL	o	6	BL	BL	o	86	x	x	o
	Hyphomicrobiaceae	1020	x	x	x	1572	x	x	x	2541	x	x	x	975	x	x	x	662	x	x	x	650	x	x	x
	Hyphomonadaceae	26	x	x	o	14	BL	BL	o	54	x	BL	o	9	BL	BL	o	11	x	BL	o	10	BL	BL	o
	Iamiaceae	7	BL	BL	o	13	BL	BL	o	15	BL	BL	o	2	BL	BL	o	3	BL	BL	o		N	N	N
	Idiomarinaceae		N	N	N		N	N	N	3	BL	BL	o		N	N	N	1	BL	BL	o	11	BL	BL	o
	Ignavibacteriaceae	539	x	x	x	678	x	x	x	788	x	x	x	425	x	x	x	213	x	x	x	113	x	x	o
	Intrasporangiaceae	18	x	BL	o	25	x	BL	o	30	BL	BL	o	11	BL	BL	o	1	BL	BL	o	20	BL	BL	o
	Isosphaeraceae	138	x	x	o	197	x	x	o	768	x	x	x	336	x	x	x	143	x	x	x	655	x	x	x
	Kiloniellaceae	9	BL	BL	o	19	BL	BL	o	38	x	BL	o	16	x	BL	o	9	x	BL	o	17	BL	BL	o
	Kineosporiaceae	14	x	BL	o	3	BL	BL	o	13	BL	BL	o	4	BL	BL	o	3	BL	BL	o		N	N	N
	Lachnospiraceae	63	x	x	o	94	x	x	o	457	x	x	o	312	x	x	x	121	x	x	o	24	x	BL	o
y	Lactobacillaceae	9	BL	BL	o	45	x	x	o	34	BL	BL	o	16	x	BL	o	13	x	x	o	3	BL	BL	o
	Legionellaceae	33	x	x	o	32	x	BL	o	115	x	x	o	42	x	x	o	23	x	x	o	199	x	x	o
	Leptospiraceae	2	BL	BL	o	2	BL	BL	o	3	BL	BL	o	4	BL	BL	o	2	BL	BL	o	7	BL	BL	o
	Leptotrichiaceae		N	N	N	4	BL	BL	o	11	BL	BL	o	4	BL	BL	o	3	BL	BL	o	1	BL	BL	o
	Leuconostocaceae	250	x	x	x	334	x	x	o	982	x	x	x	462	x	x	x	214	x	x	x	251	x	x	o
	Listeriaceae	7	BL	BL	o	12	BL	BL	o	16	BL	BL	o	5	BL	BL	o	2	BL	BL	o	7	BL	BL	o
	Litoricolaceae	2	BL	BL	o	3	BL	BL	o	3	BL	BL	o	4	BL	BL	o		N	N	N	81	x	x	o
y	Methanobacteriaceae	19	x	BL	o	25	x	BL	o	87	x	x	o	42	x	x	o	10	x	BL	o	1	BL	BL	o
	Methanocellaceae	1	BL	BL	o	1	BL	BL	o	5	BL	BL	o	5	BL	BL	o	2	BL	BL	o		N	N	N
y	Methanocorpusculaceae		N	N	N	1	BL	BL	o	1	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Methanomicrobiaceae	6	BL	BL	o	6	BL	BL	o	3	BL	BL	o	7	BL	BL	o	5	BL	BL	o		N	N	N
	Methanoregulaceae		N	N	N	4	BL	BL	o	6	BL	BL	o	5	BL	BL	o		N	N	N		N	N	N
y	Methanosaetaceae	17	x	BL	o	76	x	x	o	134	x	x	o	42	x	x	o	25	x	x	o		N	N	N
y	Methanosarcinaceae	4	BL	BL	o	5	BL	BL	o	22	BL	BL	o	8	BL	BL	o	2	BL	BL	o		N	N	N
y	Methanospirillaceae	8	BL	BL	o	3	BL	BL	o	7	BL	BL	o		N	N	N	2	BL	BL	o		N	N	N
	Methylacidiphilaceae	77	x	x	o	89	x	x	o	331	x	x	o	162	x	x	o	54	x	x	o	758	x	x	x
	Methylobacteriaceae	18	x	BL	o	31	x	BL	o	72	x	x	o	22	x	BL	o	11	x	BL	o	53	x	x	o
y	Methylococcaceae	111	x	x	o	134	x	x	o	192	x	x	o	88	x	x	o	59	x	x	o	657	x	x	x
y	Methylocystaceae	230	x	x	x	283	x	x	o	712	x	x	x	301	x	x	x	151	x	x	x	51	x	x	o
	Methylophilaceae	11	x	BL	o	14	BL	BL	o	13	BL	BL	o	8	BL	BL	o	8	x	BL	o	537	x	x	x
	Microbacteriaceae	42	x	x	o	47	x	x	o	180	x	x	o	84	x	x	o	29	x	x	o	140	x	x	o
	Micrococcaceae	31	x	x	o	202	x	x	o	63	x	BL	o	33	x	x	o	24	x	x	o	15	BL	BL	o
	Microcystaceae		N	N	N	1	BL	BL	o	2	BL	BL	o		N	N	N	2	BL	BL	o	1	BL	BL	o
	Micromonosporaceae	97	x	x	o	159	x	x	o	206	x	x	o	105	x	x	o	58	x	x	o	160	x	x	o

Ref ?	Family member	BV3 deep	Incl. BV3dp (>0.05%	Incl. BV3dp (>0.1%)	Incl. BV3dp (>1.0%)	BV3 shallow	Incl. BV3sh (>0.05%	Incl. BV3sh (>0.1%)	Incl. BV3sh (>1.0%)	BV6 deep	Incl. BV6dp (>0.05%	Incl. BV6dp (>0.1%)	Incl. BV6dp (>1.0%)	BV6 shallow	Incl. BV6sh (>0.05%	Incl. BV6sh (>0.1%)	Incl. BV6sh (>1.0%)	BV8	Incl. BV8 (>0.05%)	Incl. BV8 (>0.1%	Incl. BV8 (>1.0%	MW6 13.2-4	Incl. MW6 2-4 (>0.05%)	Incl. MW6 2-4 (>0.1%)	Incl. MW6 2-4 (1.0%)
	Micromonosporaceae	97	x	x	o	159	x	x	o	206	x	x	o	105	x	x	o	58	x	x	o	160	x	x	o
	Moraxellaceae	11	x	BL	o	33	x	BL	o	97	x	x	o	141	x	x	o	11	x	BL	o	190	x	x	o
	Moritellaceae	1	BL	BL	o		N	N	N	1	BL	BL	o	3	BL	BL	o	2	BL	BL	o	1	BL	BL	o
y	Mycobacteriaceae	34	x	x	o	44	x	x	o	84	x	x	o	39	x	x	o	12	x	BL	o	72	x	x	o
	Mycoplasmataceae	94	x	x	o	72	x	x	o	228	x	x	o	80	x	x	o	48	x	x	o	25	x	BL	o
	Myxococcaceae	81	x	x	o	69	x	x	o	422	x	x	o	164	x	x	o	26	x	x	o	6	BL	BL	o
	Nannocystaceae	29	x	x	o	31	x	BL	o	72	x	x	o	38	x	x	o	19	x	x	o	26	x	BL	o
	Neisseriaceae	47	x	x	o	46	x	x	o	142	x	x	o	39	x	x	o	16	x	x	o	262	x	x	o
	Nitriliruptoraceae		N	N	N	1	BL	BL	o	1	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Nitrosomonadaceae	9	BL	BL	o	3	BL	BL	o		N	N	N	5	BL	BL	o	4	BL	BL	o	1	BL	BL	o
	Nitrospinaceae		N	N	N		N	N	N		N	N	N	1	BL	BL	o		N	N	N		N	N	N
	Nitrospiraceae	4	BL	BL	o	6	BL	BL	o	11	BL	BL	o	7	BL	BL	o	3	BL	BL	o	228	x	x	o
y	Nocardiaceae	38	x	x	o	80	x	x	o	169	x	x	o	62	x	x	o	40	x	x	o	101	x	x	o
	Nocardioidaceae	181	x	x	o	214	x	x	o	363	x	x	o	148	x	x	o	97	x	x	o	142	x	x	o
	Nostocaceae	7	BL	BL	o	6	BL	BL	o	14	BL	BL	o	4	BL	BL	o	3	BL	BL	o	133	x	x	o
	Oceanospirillaceae	22	x	x	o	29	x	BL	o	49	x	BL	o	29	x	BL	o	28	x	x	o	1202	x	x	x
	Odoribacteraceae		N	N	N	1	BL	BL	o	4	BL	BL	o	1	BL	BL	o		N	N	N		N	N	N
	Opitutaceae	47	x	x	o	44	x	x	o	104	x	x	o	48	x	x	o	22	x	x	o	107	x	x	o
	Oscillochloridaceae		N	N	N	1	BL	BL	o	7	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Oxalobacteraceae	43	x	x	o	417	x	x	x	165	x	x	o	58	x	x	o	37	x	x	o	926	x	x	x
	Paenibacillaceae	129	x	x	o	1246	x	x	x	354	x	x	o	220	x	x	o	89	x	x	o	163	x	x	o
	Parachlamydiaceae	10	BL	BL	o	36	x	BL	o	45	x	BL	o	22	x	BL	o	5	BL	BL	o	105	x	x	o
	Pasteurellaceae		N	N	N	1	BL	BL	o	3	BL	BL	o	4	BL	BL	o	3	BL	BL	o		N	N	N
	Patulibacteraceae	1	BL	BL	o		N	N	N		N	N	N		N	N	N	1	BL	BL	o		N	N	N
y	Peptococcaceae	208	x	x	o	228	x	x	o	497	x	x	o	220	x	x	o	115	x	x	o	314	x	x	o
	Peptostreptococcaceae	611	x	x	x	507	x	x	x	2849	x	x	x	799	x	x	x	549	x	x	x	18	BL	BL	o
	Phyllobacteriaceae	120	x	x	o	163	x	x	o	209	x	x	o	90	x	x	o	72	x	x	o	17	BL	BL	o
	Piscirickettsiaceae	24	x	x	o	23	x	BL	o	94	x	x	o	15	BL	BL	o	12	x	BL	o	179	x	x	o
	Planctomycetaceae	157	x	x	o	235	x	x	o	677	x	x	o	240	x	x	o	133	x	x	x	862	x	x	x
	Planococcaceae	43	x	x	o	1253	x	x	x	143	x	x	o	270	x	x	o	26	x	x	o	349	x	x	o
	Polyangiaceae	111	x	x	o	92	x	x	o	353	x	x	o	115	x	x	o	65	x	x	o	298	x	x	o
y	Porphyromonadaceae	285	x	x	x	250	x	x	o	827	x	x	x	380	x	x	x	126	x	x	o	2	BL	BL	o
	Promicromonosporaceae		N	N	N	1	BL	BL	o	2	BL	BL	o	2	BL	BL	o		N	N	N	1	BL	BL	o
y	Propionibacteriaceae	9	BL	BL	o	1	BL	BL	o	25	BL	BL	o	16	x	BL	o		N	N	N		N	N	N
	Pseudanabaenaceae	4	BL	BL	o	5	BL	BL	o	6	BL	BL	o	2	BL	BL	o	4	BL	BL	o	369	x	x	o
	Pseudoalteromonadaceae	10	BL	BL	o	20	x	BL	o	55	x	BL	o	20	x	BL	o	12	x	BL	o	52	x	x	o
	Pseudomonadaceae	63	x	x	o	219	x	x	o	63	x	BL	o	32	x	x	o	31	x	x	o	460	x	x	x
	Pseudonocardiaceae	161	x	x	o	244	x	x	o	634	x	x	o	308	x	x	x	166	x	x	x	460	x	x	x
	Psychromonadaceae	1	BL	BL	o	1	BL	BL	o	3	BL	BL	o	4	BL	BL	o		N	N	N	2	BL	BL	o
	Puniceicoccaceae	12	x	BL	o	12	BL	BL	o	36	x	BL	o	17	x	BL	o	2	BL	BL	o	5	BL	BL	o
	Rhabdochlamydiaceae	109	x	x	o	130	x	x	o	294	x	x	o	123	x	x	o	57	x	x	o	3	BL	BL	o
	Rhizobiaceae	81	x	x	o	154	x	x	o	177	x	x	o	77	x	x	o	53	x	x	o	145	x	x	o
	Rhodobacteraceae	59	x	x	o	83	x	x	o	112	x	x	o	59	x	x	o	42	x	x	o	303	x	x	o
	Rhodobiaceae	18	x	BL	o	27	x	BL	o	28	BL	BL	o	19	x	BL	o	15	x	x	o	1	BL	BL	o
y	Rhodocyclaceae	124	x	x	o	184	x	x	o	233	x	x	o	74	x	x	o	50	x	x	o	851	x	x	x
	Rhodospirillaceae	392	x	x	x	483	x	x	x	1235	x	x	x	457	x	x	x	236	x	x	x	1138	x	x	x

Ref ?	Family member	BV3 deep	Incl. BV3dp (>0.05%	Incl. BV3dp (>0.1%)	Incl. BV3dp (>1.0%)	BV3 shallow	Incl. BV3sh (>0.05%	Incl. BV3sh (>0.1%)	Incl. BV3sh (>1.0%)	BV6 deep	Incl. BV6dp (>0.05%	Incl. BV6dp (>0.1%)	Incl. BV6dp (>1.0%)	BV6 shallow	Incl. BV6sh (>0.05%	Incl. BV6sh (>0.1%)	Incl. BV6sh (>1.0%)	BV8	Incl. BV8 (>0.05%)	Incl. BV8 (>0.1%	Incl. BV8 (>1.0%	MW6 13.2-4	Incl. MW6 2-4 (>0.05%)	Incl. MW6 2-4 (>0.1%)	Incl. MW6 2-4 (1.0%)
	Rhodospirillaceae	392	x	x	x	483	x	x	x	1235	x	x	x	457	x	x	x	236	x	x	x	1138	x	x	x
	Rhodothermaceae	16	x	BL	o	23	x	BL	o	84	x	x	o	35	x	x	o	4	BL	BL	o	3577	x	x	x
	Rickettsiaceae	27	x	x	o	46	x	x	o	149	x	x	o	66	x	x	o	36	x	x	o	182	x	x	o
	Rikenellaceae	3	BL	BL	o	4	BL	BL	o		N	N	N	2	BL	BL	o		N	N	N		N	N	N
	Rivulariaceae	1	BL	BL	o	2	BL	BL	o	4	BL	BL	o	6	BL	BL	o	1	BL	BL	o	771	x	x	x
	Rubrobacteraceae	2	BL	BL	o	4	BL	BL	o	10	BL	BL	o	5	BL	BL	o	2	BL	BL	o		N	N	N
	Ruminococcaceae	22	x	x	o	38	x	BL	o	102	x	x	o	50	x	x	o	28	x	x	o	57	x	x	o
	Saccharospirillaceae	7	BL	BL	o	3	BL	BL	o	6	BL	BL	o		N	N	N	1	BL	BL	o	66	x	x	o
	Sanguibacteraceae	1	BL	BL	o	2	BL	BL	o	8	BL	BL	o	2	BL	BL	o		N	N	N	4	BL	BL	o
	Saprospiraceae	54	x	x	o	90	x	x	o	88	x	x	o	45	x	x	o	41	x	x	o	428	x	x	o
y	Shewanellaceae	15	x	BL	o	11	BL	BL	o	35	x	BL	o	13	BL	BL	o	8	x	BL	o	889	x	x	x
	Sinobacteraceae	194	x	x	o	282	x	x	o	381	x	x	o	180	x	x	o	126	x	x	o	233	x	x	o
	Solibacteraceae	37	x	x	o	77	x	x	o	316	x	x	o	127	x	x	o	37	x	x	o	548	x	x	x
	Solirubrobacteraceae	54	x	x	o	67	x	x	o	133	x	x	o	51	x	x	o	32	x	x	o	17	BL	BL	o
	Sphingobacteriaceae	1317	x	x	x	1400	x	x	x	5294	x	x	x	1763	x	x	x	536	x	x	x	505	x	x	x
	Sphingomonadaceae	715	x	x	x	925	x	x	x	1484	x	x	x	568	x	x	x	395	x	x	x	1104	x	x	x
	Spirochaetaceae	214	x	x	x	234	x	x	o	516	x	x	o	229	x	x	o	77	x	x	o	57	x	x	o
	Sporichthyaceae		N	N	N		N	N	N		N	N	N		N	N	N		N	N	N	1	BL	BL	o
	Sporolactobacillaceae		N	N	N		N	N	N	1	BL	BL	o		N	N	N		N	N	N		N	N	N
	Staphylococcaceae	13	x	BL	o	17	BL	BL	o	33	BL	BL	o	19	x	BL	o	9	x	BL	o	15	BL	BL	o
y	Streptococcaceae	9	BL	BL	o	21	x	BL	o	37	x	BL	o	21	x	BL	o	5	BL	BL	o	9	BL	BL	o
	Streptomycetaceae	28	x	x	o	65	x	x	o	118	x	x	o	59	x	x	o	28	x	x	o	206	x	x	o
	Streptosporangiaceae	26	x	x	o	31	x	BL	o	107	x	x	o	48	x	x	o	16	x	x	o	38	x	BL	o
	Symbiobacteriaceae	53	x	x	o	20	x	BL	o	51	x	BL	o	35	x	x	o	5	BL	BL	o		N	N	N
	Synechococcaceae		N	N	N		N	N	N	3	BL	BL	o		N	N	N		N	N	N		N	N	N
y	Synergistaceae	12	x	BL	o	16	BL	BL	o	69	x	BL	o	27	x	BL	o	9	x	BL	o	2	BL	BL	o
y	Syntrophaceae	89	x	x	o	140	x	x	o	221	x	x	o	116	x	x	o	46	x	x	o	144	x	x	o
y	Syntrophobacteraceae	34	x	x	o	55	x	x	o	85	x	x	o	41	x	x	o	28	x	x	o	5	BL	BL	o
	Syntrophomonadaceae	8	BL	BL	o	17	BL	BL	o	61	x	BL	o	34	x	x	o	15	x	x	o	2	BL	BL	o
	Thermaceae	10	BL	BL	o	10	BL	BL	o	33	BL	BL	o	12	BL	BL	o	10	x	BL	o	4	BL	BL	o
	Thermoactinomycetaceae	25	x	x	o	46	x	x	o	106	x	x	o	34	x	x	o	23	x	x	o	2	BL	BL	o
	Thermoanaerobacteraceae	247	x	x	x	307	x	x	o	1033	x	x	x	416	x	x	x	155	x	x	x	288	x	x	o
	Thermococcaceae	1	BL	BL	o		N	N	N		N	N	N		N	N	N		N	N	N		N	N	N
y	Thermodesulfobacteriaceae	61	x	x	o	43	x	x	o	177	x	x	o	94	x	x	o	50	x	x	o	1	BL	BL	o
	Thermogemmatisporaceae	142	x	x	o	163	x	x	o	510	x	x	o	219	x	x	o	105	x	x	o	277	x	x	o
	Thermomonosporaceae	58	x	x	o	41	x	x	o	178	x	x	o	56	x	x	o	12	x	BL	o	4	BL	BL	o
	Thermoproteaceae	1	BL	BL	o	2	BL	BL	o	14	BL	BL	o	6	BL	BL	o	5	BL	BL	o		N	N	N
	Thermotogaceae	41	x	x	o	78	x	x	o	202	x	x	o	100	x	x	o	41	x	x	o	249	x	x	o
	Thiotrichaceae	13	x	BL	o	9	BL	BL	o	22	BL	BL	o	9	BL	BL	o	3	BL	BL	o	337	x	x	o
	Tsukamurellaceae	7	BL	BL	o		N	N	N	2	BL	BL	o	6	BL	BL	o	1	BL	BL	o		N	N	N
	Veillonellaceae	259	x	x	x	371	x	x	o	955	x	x	x	471	x	x	x	240	x	x	x	274	x	x	o
	Verrucomicrobiaceae	72	x	x	o	144	x	x	o	105	x	x	o	38	x	x	o	54	x	x	o	1033	x	x	x
	Vibrionaceae		N	N	N	1	BL	BL	o	3	BL	BL	o	5	BL	BL	o		N	N	N	4	BL	BL	o
	Waddliaceae	140	x	x	o	205	x	x	o	451	x	x	o	176	x	x	o	91	x	x	o	3	BL	BL	o
	Xanthobacteraceae	162	x	x	o	225	x	x	o	461	x	x	o	201	x	x	o	114	x	x	o	150	x	x	o
y	Xanthomonadaceae	287	x	x	x	369	x	x	o	454	x	x	o	195	x	x	o	101	x	x	o	424	x	x	o

Ref ?	Family member	BV3 deep	Incl. BV3dp (>0.05%	Incl. BV3dp (>0.1%)	Incl. BV3dp (>1.0%)	BV3 shallow	Incl. BV3sh (>0.05%	Incl. BV3sh (>0.1%)	Incl. BV3sh (>1.0%)	BV6 deep	Incl. BV6dp (>0.05%	Incl. BV6dp (>0.1%)	Incl. BV6dp (>1.0%)	BV6 shallow	Incl. BV6sh (>0.05%	Incl. BV6sh (>0.1%)	Incl. BV6sh (>1.0%)	BV8	Incl. BV8 (>0.05%)	Incl. BV8 (>0.1%	Incl. BV8 (>1.0%	MW6 13.2-4	Incl. MW6 2-4 (>0.05%)	Incl. MW6 2-4 (>0.1%)	Incl. MW6 2-4 (1.0%)
	Veillonellaceae	259	x	x	x	371	x	x	o	955	x	x	x	471	x	x	x	240	x	x	x	274	x	x	o
	Verrucomicrobiaceae	72	x	x	o	144	x	x	o	105	x	x	o	38	x	x	o	54	x	x	o	1033	x	x	x
	Vibrionaceae		N	N	N	1	BL	BL	o	3	BL	BL	o	5	BL	BL	o		N	N	N	4	BL	BL	o
	Waddliaceae	140	x	x	o	205	x	x	o	451	x	x	o	176	x	x	o	91	x	x	o	3	BL	BL	o
	Xanthobacteraceae	162	x	x	o	225	x	x	o	461	x	x	o	201	x	x	o	114	x	x	o	150	x	x	o
y	Xanthomonadaceae	287	x	x	x	369	x	x	o	454	x	x	o	195	x	x	o	101	x	x	o	424	x	x	o
	Grand Total	####				38055				####				30586				12762				###			
	Cutoff (Family lvl) = 0.05%	10				19				35				15				6				23			
	Cutoff (Family lvl) = 0.1%	21		x		38				70				31				13				45			
	Cutoff (Family lvl) = 1.0%	210				381				697				306				128				451			
	Updated grand total		20692	20292	14133		37571	36750	26093		68760	67709	47333		30204	29700	20424		12611	12348	8468		44759	44450	32126
	Not found in sample (N)	198	132	104	23	205		95	17	211		106	23	195		106	22	189		105	23	181		101	32
	Below cutoff 0.1% (BL)			3.4	32.7			3.4	31.4			2.9	32.1			2.9	33.2			3.2	33.6			1.5	28.8
38	**Reference table**		27	22	9			22				25				25				22				20	
	Dhc	0.024		10.5		0.03416108				0.044				0.03596				0.047				0.00			

Appendix D2: Example of NCBI database filtering of Illumina raw data (screenshot)



Kingdom	Phylum	Class	Order	Family	Genus	Species	num_hits	%_hits	Reject	_	actual_superkingdom	actual_kingdom	actual_phylum	actual_class	actual_order	NCBI_TaxID
Unclassified							237447	76.311	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria							4777	1.535	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Proteobacteria	Gammaproteobacteria					3464	1.113	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Rhodothermaceae	Salinibacter		3332	1.071			Bacteria	-	Bacteroidetes	0	Bacteroidetes	1855405
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales				2453	0.788	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Kushneria	aurantia	2321	0.746			Bacteria	-	Proteobacteria	Gammaprote	Oceanospiril	1395977
Bacteria	Proteobacteria						1779	0.572	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Cyanobacteria	Nostocophycideae	Nostocales	Nostocaceae			1176	0.378	x		Bacteria	-	Cyanobacteria	0	Nostocales	2035978
Bacteria	Cyanobacteria	Oscillatoriophycideae	Chroococcales				1125	0.362	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Verrucomicrobia	Pedosphaerae	Pedosphaerales	Pedosphaeraceae	Pedosphaera		929	0.299	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	sediminis	925	0.297			Bacteria	-	Proteobacteria	Gammaprote	Oceanospiril	1395977
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella		874	0.281			Bacteria	-	Proteobacteria	Gammaprote	Alteromonad	1573826
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae			830	0.267	x		Bacteria	-	Bacteroidetes	Flavobacterii	Flavobacteria	449462
Bacteria	Cyanobacteria						783	0.252	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Proteobacteria	Betaproteobacteria					768	0.247	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Verrucomicrobia	Methylacidiphilae	Methylacidiphilales	Methylacidiphilaceae	Candidatus Methylacidiphilum		758	0.244			Bacteria	-	Verrucomicrobia	Methylacidip	Methylacidip	511745
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	bombi	731	0.235			Bacteria	-	Actinobacteria	Actinobacteri	Bifidobacteri	240233
Bacteria	Cyanobacteria	Nostocophycideae	Stigonematales	Rivulariaceae	Calothrix	parietina	729	0.234			Bacteria	-	Cyanobacteria	0	Nostocales	1186
Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	flavus	729	0.234			Bacteria	-	Verrucomicrobia	Spartobacter	Chthoniobac	1921511
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	variicola	679	0.218			Bacteria	-	Proteobacteria	Gammaprote	Enterobacter	1903434
Bacteria	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Edaphobacter	modestus	636	0.204			Bacteria	-	Acidobacteria	Acidobacterii	Acidobacteri.	1078830
Bacteria	Proteobacteria	Gammaproteobacteria	Thiohalorhabdales	Thiohalorhabdaceae	Thiohalorhabdus	denitrificans	588	0.189	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas		573	0.184			Bacteria	-	Proteobacteria	Gammaprote	Oceanospiril	1395977
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae			567	0.182	x		Bacteria	-	Proteobacteria	Betaproteoba	Burkholderia	12916
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Halorhodospira	halochloris	552	0.177			Bacteria	-	Proteobacteria	Gammaprote	Chromatiales	1765964
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Marinospirillum		549	0.176			Bacteria	-	Proteobacteria	Gammaprote	Oceanospiril	515417
Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	Candidatus Solibacter		548	0.176			Bacteria	-	Acidobacteria	Solibacteres	Solibacterale	332162
Bacteria	Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	Singulisphaera		540	0.174			Bacteria	-	Planctomycetes	Planctomyce	Planctomyce	1511635
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales				487	0.157	x		NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH	NO_MATCH
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Marinomonas	basaltis	477	0.153			Bacteria	-	Proteobacteria	Gammaprote	Oceanospiril	515417
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Peptoniphilus	coxii	441	0.142			Bacteria	-	Firmicutes	Clostridia	Clostridiales	114627
Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces		430	0.138			Bacteria	-	Planctomycetes	Planctomyce	Planctomyce	265487
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Chromatium	weissei	427	0.137			Bacteria	-	Proteobacteria	Gammaprote	Chromatiales	85072
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Amoebophilaceae	Candidatus Amoebophilus	asiaticus	405	0.13			Bacteria	-	Bacteroidetes	Cytophagia	Cytophagale:	281119
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes		392	0.126			Bacteria	-	Proteobacteria	Alphaproteot	Rhizobiales	204472
Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	Thermoanaerobacteraceae			388	0.125	x		Bacteria	-	Firmicutes	Clostridia	Thermoanae	1648500
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter		383	0.123			Bacteria	-	Verrucomicrobia	Verrucomicr(	Verrucomicr(	1383070
Bacteria	Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	Gemmata		378	0.121			Bacteria	-	Planctomycetes	Planctomyce	Planctomyce	1914234
Bacteria	Cyanobacteria	Synechococcophycideae	Pseudanabaenales	Pseudanabaenaceae	Arthronema	africanum	367	0.118			Bacteria	-	Cyanobacteria	0	Synechococc	1620700
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	kwanguangensi	355	0.114			Bacteria	-	Bacteroidetes	Sphingobact	Sphingobact	1773450

Appendix D3: qPCR raw data- *Dehalococcoides* target in environmental samples

Dhc = 72bp	Conc = ng/uL			>H2O Conc = ng/uL			Conc (ng/g)= ng/uL*ratio Conversion ratio: 2 uL = 9.9 g)			Copy #/g = (conc ng/uL * 6.0221x10^23 molec/mol) / (ampl length * 660 g/mol * 1x10^9 ng/g)		
Sample	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
01. BW6	-	-	-									
02. BW6	-	5.34E-09	-		5.34E-09			1.08E-09			1.37E+01	
03. BW6	-	-	-									
04. BW8	-	-	-									
05. BW8	-	-	-									
06. BW8	-	-	-									
07. BW3	-	-	-									
08. BW3	-	2.08E-09	-									
09. BW3	-	-	-									
10. BW3	-	-	-									
11. BW3	-	-	-									
12. BW3	-	-	-									
13. BW6	1.10E-10	1.49E-07	8.70E-09		1.49E-07	8.70E-09		3.01E-08	1.76E-09		3.81E+02	2.23E+01
14. BW6	5.26E-08	9.60E-08	2.96E-08	5.26E-08	9.60E-08	2.96E-08	1.06E-08	1.94E-08	5.98E-09	1.35E+02	2.46E+02	7.58E+01
15. BW6	2.42E-10	3.36E-09	3.00E-08		3.36E-09	3.00E-08		6.79E-10	6.06E-09		8.60E+00	7.68E+01
2013 MW4 0"	2.35E-09	1.76E-09	4.33E-10									
2013 MW4 2"	5.07E-09	7.33E-09	4.09E-09	5.07E-09	7.33E-09	4.09E-09	1.02E-09	1.48E-09	8.26E-10	1.30E+01	1.88E+01	1.05E+01
2013 MW4 4"	1.65E-09	8.01E-10	1.54E-09									
2013 MW4 6"	6.06E-09	2.54E-09	4.33E-09	6.06E-09		4.33E-09	1.22E-09		8.75E-10	1.55E+01		1.11E+01
2013 MW4 8"	4.65E-09	9.38E-10	4.31E-09	4.65E-09		4.31E-09	9.39E-10		8.71E-10	1.19E+01		1.10E+01
2013 MW4 10"	2.70E-09	3.27E-09	4.43E-09	2.70E-09	3.27E-09	4.43E-09	5.45E-10	6.61E-10	8.95E-10	6.91E+00	8.37E+00	1.13E+01
2013 BW4 0"	4.42E-09	2.18E-09	1.44E-08	4.42E-09		1.44E-08	8.93E-10		2.91E-09	1.13E+01		3.69E+01
2013 BW4 2"	2.46E-09	1.63E-09	1.77E-09									
2013 BW4 4"	1.59E-09	3.62E-09	3.90E-09		3.62E-09	3.90E-09		7.31E-10	7.88E-10		9.27E+00	9.98E+00
Dhc1	3.70E-04	3.70E-04	3.70E-04	3.70E-04	3.70E-04	3.70E-04	7.47E-05	7.47E-05	7.47E-05	9.47E+05	9.47E+05	9.47E+05
H2O	7.62E-10	2.67E-09	4.48E-09	*2.64E-09*			5.33E-10			6.75E+00		
2013 BW4 6"	4.43E-08	3.82E-08	3.71E-08	4.43E-08	3.82E-08	3.71E-08	8.95E-09	7.72E-09	7.49E-09	1.13E+02	9.78E+01	9.50E+01
2013 BW4 8"	3.93E-08	4.04E-08	5.06E-08	3.93E-08	4.04E-08	5.06E-08	7.94E-09	8.16E-09	1.02E-08	1.01E+02	1.03E+02	1.30E+02
2013 BW4 10"	3.97E-08	4.01E-08	3.86E-08	3.97E-08	4.01E-08	3.86E-08	8.02E-09	8.10E-09	7.80E-09	1.02E+02	1.03E+02	9.88E+01
2013 MW6 0"	3.94E-08	3.98E-08	4.01E-08	3.94E-08	3.98E-08	4.01E-08	7.96E-09	8.04E-09	8.10E-09	1.01E+02	1.02E+02	1.03E+02
2013 MW6 2"	4.10E-08	3.88E-08	3.52E-08	4.10E-08	3.88E-08		8.28E-09	7.84E-09		1.05E+02	9.93E+01	
2013 MW6 4"	4.08E-08	4.62E-08	4.12E-08	4.08E-08	4.62E-08	4.12E-08	8.24E-09	9.33E-09	8.32E-09	1.04E+02	1.18E+02	1.05E+02
2013 MW6 6"	3.96E-08	3.63E-08	4.45E-08	3.96E-08		4.45E-08	8.00E-09		8.99E-09	1.01E+02		1.14E+02
2013 MW6 8"	3.49E-08	3.52E-08	3.80E-08			3.80E-08			7.68E-09			9.73E+01
2013 MW6 10"	4.04E-08	3.99E-08	3.73E-08	4.04E-08	3.99E-08	3.73E-08	8.16E-09	8.06E-09	7.54E-09	1.03E+02	1.02E+02	9.55E+01
2015 MW4 0"	3.33E-08	4.14E-08	3.97E-08		4.14E-08	3.97E-08		8.36E-09	8.02E-09		1.06E+02	1.02E+02

2015 MW4 4"	3.61E-08	4.39E-08	3.86E-08		4.39E-08	3.86E-08		8.87E-09	7.80E-09		1.12E+02	9.88E+01
2015 MW4 6"	3.89E-08	4.45E-08	4.52E-08	3.89E-08	4.45E-08	4.52E-08	7.86E-09	8.99E-09	9.13E-09	9.96E+01	1.14E+02	1.16E+02
2015 MW4 8"	4.10E-08	3.65E-08	4.11E-08	4.10E-08		4.11E-08	8.28E-09		8.30E-09	1.05E+02		1.05E+02
2015 MW4 10"	4.04E-08	4.05E-08	3.67E-08	4.04E-08	4.05E-08	3.67E-08	8.16E-09	8.18E-09	7.41E-09	1.03E+02	1.04E+02	9.40E+01
2015 BW4 0'	3.87E-08	3.83E-08	4.10E-08	3.87E-08	3.83E-08	4.10E-08	7.82E-09	7.74E-09	8.28E-09	9.91E+01	9.81E+01	1.05E+02
2015 BW4 2'	4.37E-08	4.47E-08	3.68E-08	4.37E-08	4.47E-08	3.68E-08	8.83E-09	9.03E-09	7.43E-09	1.12E+02	1.14E+02	9.42E+01
2015 BW4 4'	4.17E-08	4.67E-08	4.34E-08	4.17E-08	4.67E-08	4.34E-08	8.42E-09	9.43E-09	8.77E-09	1.07E+02	1.20E+02	1.11E+02
2015 BW4 6"	3.87E-08	4.14E-08	4.06E-08	3.87E-08	4.14E-08	4.06E-08	7.82E-09	8.36E-09	8.20E-09	9.91E+01	1.06E+02	1.04E+02
2015 BW4 10"	3.97E-08	4.03E-08	3.63E-08	3.97E-08	4.03E-08		8.02E-09	8.14E-09		1.02E+02	1.03E+02	
2015 MW6 0"	3.81E-08	3.47E-08	4.49E-08	3.81E-08		4.49E-08	7.70E-09		9.07E-09	9.75E+01		1.15E+02
2015 MW6 2"	3.78E-08	4.18E-08	3.65E-08	3.78E-08	4.18E-08		7.64E-09	8.44E-09		9.68E+01	1.07E+02	
2015 MW6 4"	4.11E-08	4.23E-08	3.85E-08	4.11E-08	4.23E-08	3.85E-08	8.30E-09	8.55E-09	7.78E-09	1.05E+02	1.08E+02	9.86E+01
2015 MW6 6"	4.11E-08	3.81E-08	3.92E-08	4.11E-08	3.81E-08	3.92E-08	8.30E-09	7.70E-09	7.92E-09	1.05E+02	9.75E+01	1.00E+02
2015 MW6 8"	4.15E-08	3.51E-08	4.39E-08	4.15E-08		4.39E-08	8.38E-09		8.87E-09	1.06E+02		1.12E+02
DHC1												
H2O	4.00E-08	3.40E-08	3.60E-08	*3.67E-08*			7.41E-09			9.39E+01		

Appendix D4: qPCR raw data- *tceA* target in environmental samples

tceA = 67bp	Conc = ng/uL			>H2O Conc = ng/uL			Conc (ng/g)= ng/uL*ratio Conversion ratio: 5 mL/2 uL = 9.9 g/ "X")			Copy #/g (conc ng/uL * 6.0221x10^23 molec/mol) / (ampl length * 660 g/mol * 1x10^9 ng/g)		
Sample	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
01. BW6												
02. BW6												
03. BW6												
04. BW8												
05. BW8												
06. BW8												
07. BW3												
08. BW3												
09. BW3												
10. BW3												
11. BW3												
12. BW3												
13. BW6	1.76E-10	5.57E-10	3.63E-10									
14. BW6		1.49E-10	2.13E-10									
15. BW6												
2013 MW4 0"	3.56E-10	1.17E-09	4.33E-09		1.17E-09	4.33E-09		2.4E-10	8.7E-10		3.219	11.913
2013 MW4 2"	1.32E-09	1.52E-09	7.55E-10	1.32E-09	1.52E-09		2.7E-10	3.1E-10		3.632	4.182	
2013 MW4 4"		1.03E-09	1.63E-09		1.03E-09	1.63E-09		2.1E-10	3.3E-10		2.834	4.484
2013 MW4 6"	6.36E-10	1.97E-09	1.56E-09		1.97E-09	1.56E-09		4E-10	3.2E-10		5.420	4.292
2013 MW4 8"	5.75E-09	2.14E-09	2.67E-09	5.75E-09	2.14E-09	2.67E-09	1.2E-09	4.3E-10	5.4E-10	15.819	5.888	7.346
2013 MW4 10"	1.02E-09	1.25E-09	1.33E-09	1.02E-09	1.25E-09	1.33E-09	2.1E-10	2.5E-10	2.7E-10	2.806	3.439	3.659
2013 BW4 0"	1.05E-09	3.36E-10		1.05E-09			2.1E-10			2.889		
2013 BW4 2"	2.28E-09	1.4E-09	1.86E-09	2.28E-09	1.4E-09	1.86E-09	4.6E-10	2.8E-10	3.8E-10	6.273	3.852	5.117
2013 BW4 4"	1.84E-09	2.93E-09	2.15E-09	1.84E-09	2.93E-09	2.15E-09	3.7E-10	5.9E-10	4.3E-10	5.062	8.061	5.915
2013 BW4 6"												
2013 BW4 8"	1.62E-09			1.62E-09			3.3E-10			4.457		
2013 BW4 10"												
2013 MW6 0"												
2013 MW6 2"	5.65E-10		7.58E-10									
2013 MW6 4"			5.5E-10									
2013 MW6 6"	5.51E-10											
2013 MW6 8"			7.83E-10									
2013 MW6 10"	6.22E-10											
2015 MW4 0"	2.5E-09	2.74E-09	7.89E-10	2.5E-09	2.74E-09		5.1E-10	5.5E-10		6.878	7.538	
2015 MW4 4"	1.28E-09			1.28E-09			2.6E-10			3.522		

2015 MW4 6"	9.88E-10		1.35E-09			1.35E-09			2.7E-10			3.714
2015 MW4 8"	8.28E-10	2.71E-09	4.71E-09		2.71E-09	4.71E-09		5.5E-10	9.5E-10		7.456	12.958
2015 MW4 10"	2.48E-09	2.18E-09	1.34E-09	2.48E-09	2.18E-09	1.34E-09	5E-10	4.4E-10	2.7E-10	6.823	5.998	3.687
2015 BW4 0"	1.47E-09	8.02E-10	1.17E-09	1.47E-09			3E-10			4.044		
2015 BW4 2"	5.16E-10											
2015 BW4 4"	2.58E-09		7.15E-10	2.58E-09			5.2E-10			7.098		
2015 BW4 6"	4.05E-09	7.21E-10	2.03E-09	4.05E-09		2.03E-09	8.2E-10		4.1E-10	11.142		5.585
2015 BW4 10"	2.76E-09	8.21E-10	1.04E-09	2.76E-09			5.6E-10			7.593		
2015 MW6 0"	2.28E-09	2.52E-09	3.02E-09	2.28E-09	2.52E-09	3.02E-09	4.6E-10	5.1E-10	6.1E-10	6.273	6.933	8.309
2015 MW6 2"	3.25E-09	1.51E-09	1.35E-09	3.25E-09	1.51E-09	1.35E-09	6.6E-10	3.1E-10	2.7E-10	8.941	4.154	3.714
2015 MW6 4"	1.88E-09		6.59E-10	1.88E-09			3.8E-10			5.172		
2015 MW6 6"	1.4E-09	1.5E-09	1.86E-09	1.4E-09	1.5E-09	1.86E-09	2.8E-10	3E-10	3.8E-10	3.852	4.127	5.117
2015 MW6 8"	1.73E-09	1.57E-09	2.14E-09	1.73E-09	1.57E-09	2.14E-09	3.5E-10	3.2E-10	4.3E-10	4.760	4.319	5.888
h2o 2	1.83E-09	1.60E-10	1.61E-09									
h2o 1	4.37E-10	4.32E-10	1.71E-09									

Appendix D5: qPCR raw data- *vcrA* target in environmental samples

vcrA = 65bp		Conc = ng/uL			>H2O Conc = ng/uL			Conc (ng/g)= ng/uL*ratio Conversion ratio: 5 mL/2 uL = 9.9 g/ "X")			Copy #/g (conc ng/uL * 6.0221x10^23 molec/mol) / (ampl length * 660 g/mol * 1x10^9 ng/g)	
Sample	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
01. BW6												
02. BW6												
03. BW6												
04. BW8		3.58E-09			3.58E-09			7.23E-10			10.144	
05. BW8												
06. BW8		4.16E-09	3.08E-10		4.16E-09			8.4E-10			11.792	
07. BW3												
08. BW3												
09. BW3												
10. BW3												
11. BW3												
12. BW3												
13. BW6	1.06E-09	1.33E-09	8.53E-10									
14. BW6												
15. BW6												
2013 MW4 0"		9.82E-10										
2013 MW4 2"		6.88E-09	1.41E-09		6.88E-09			1.39E-09			19.522	
2013 MW4 4"	1.43E-09		3.79E-10									
2013 MW4 6"	1.48E-09	9.76E-10										
2013 MW4 8"	8.02E-10		4.16E-10									
2013 MW4 10"	2.13E-09		7.35E-10	2.13E-09			4.31E-10			6.052		
2013 BW4 0"			1.04E-09									
2013 BW4 2"	8.01E-10	1.61E-09										
2013 BW4 4"			4.33E-09			4.33E-09			8.74E-10			12.265
2013 BW4-6	3.04E-09	6.77E-10	2.99E-09	3.04E-09		2.99E-09	6.14E-10		6.04E-10	8.621		8.479
2013 BW4-8	3.23E-09	1.4E-09	1.26E-09	3.23E-09			6.53E-10			9.160		
2013 BW4-10	1.76E-09	4.9E-09		1.76E-09	4.9E-09		3.56E-10	9.9E-10		4.991	13.896	
2013 MW6-0	5.89E-09	5.33E-09	5.22E-09	5.89E-09	5.33E-09	5.22E-09	1.19E-09	1.08E-09	1.05E-09	16.703	15.115	14.803
2013 MW6-2	1.91E-09	4.99E-10	3.86E-09	1.91E-09		3.86E-09	3.86E-10		7.8E-10	5.417		10.946
2013 MW6-4	2.14E-09	2.43E-09		2.14E-09	2.43E-09		4.32E-10	4.91E-10		6.069	6.891	
2013 MW6-6	1.93E-09	4.41E-09	6.22E-09	1.93E-09	4.41E-09	6.22E-09	3.9E-10	8.91E-10	1.26E-09	5.473	12.506	17.639
2013 MW6-8	1.91E-09	6.05E-09		1.91E-09	6.05E-09		3.86E-10	1.22E-09		5.417	17.157	
2013 MW6-10		4.23E-09	4.35E-10		4.23E-09			8.55E-10			11.996	
2015 MW4-0	9.86E-09		4.49E-09	9.86E-09		4.49E-09	1.99E-09		9.07E-10	27.962		12.733
2015 MW4-4	6.89E-09	4.58E-09	5.4E-09	6.89E-09	4.58E-09	5.4E-09	1.39E-09	9.25E-10	1.09E-09	19.539	12.988	15.314

2015 MW4-6	1.37E-09	2.33E-09	4.53E-09		2.33E-09	4.53E-09		4.71E-10	9.15E-10		6.608	12.846
2015 MW4-8	1.69E-09	4.64E-09	3.5E-09	1.69E-09	4.64E-09	3.5E-09	3.41E-10	9.37E-10	7.07E-10	4.793	13.158	9.926
2015 MW4-10	5.28E-09		3.31E-09	5.28E-09		3.31E-09	1.07E-09		6.69E-10	14.973		9.387
2015 BW4-0	5.03E-09	2.3E-09	3.89E-09	5.03E-09	2.3E-09	3.89E-09	1.02E-09	4.65E-10	7.86E-10	14.264	6.522	11.032
2015 BW4-2	2.1E-08	6.89E-09	3.55E-09	2.1E-08	6.89E-09	3.55E-09	4.24E-09	1.39E-09	7.17E-10	59.553	19.539	10.067
2015 BW4-4	5.44E-09	2.67E-09	3.32E-09	5.44E-09	2.67E-09	3.32E-09	1.1E-09	5.39E-10	6.71E-10	15.427	7.572	9.415
2015 BW4-10	4.77E-09		5.38E-09	4.77E-09		5.38E-09	9.64E-10		1.09E-09	13.527		15.257
2015 MW6-0	4.02E-09	2.13E-09	3.54E-09	4.02E-09	2.13E-09	3.54E-09	8.12E-10	4.3E-10	7.15E-10	11.400	6.040	10.039
2015 MW6-2	1.14E-09	7.57E-09	6.97E-09		7.57E-09	6.97E-09		1.53E-09	1.41E-09		21.467	19.766
2015 MW6-4	4.35E-09	3.42E-09	5.08E-09	4.35E-09	3.42E-09	5.08E-09	8.79E-10	6.91E-10	1.03E-09	12.336	9.699	14.406
2015 MW6-6	3.63E-09	1.03E-09	4.32E-09	3.63E-09		4.32E-09	7.33E-10		8.73E-10	10.294		12.251
2015 MW6-8	2.7E-09	2.48E-09	2.74E-09	2.7E-09	2.48E-09	2.74E-09	5.45E-10	5.01E-10	5.54E-10	7.657	7.033	7.770
~~2015 MW6-10~~	5.32E-10	1.04E-09										
h2o	1.95E-09	1.61E-09	1.38E-09									

Appendix D6: qPCR raw data- *bvcA* target in environmental samples

				Conc (ng/g)= ng/uL*ratio Conversion ratio: 5 mL/2 uL = 9.9 g/ "X")			Copy #/g (conc ng/uL * 6.0221x10^23 molec/mol) / (ampl length * 660 g/mol * 1x10^9 ng/g)		
839bp		Conc = ng/uL							
Sample	R1	R2	R3	R1	R2	R3	R1	R2	R3
01. BW6									
02. BW6									
03. BW6									
04. BW8									
05. BW8									
06. BW8									
07. BW3									
08. BW3									
09. BW3									
10. BW3									
11. BW3									
12. BW3									
13. BW6									
14. BW6									
15. BW6									
2013 MW4 0"									
2013 MW4 2"									
2013 MW4 4"									
2013 MW4 6"									
2013 MW4 8"									
2013 MW4 10"									
2013 BW4 0"									
2013 BW4 2"									
2013 BW4 4"									
2013 BW4 6"									
2013 BW4 8"									
2013 BW4 10"									
2013 MW6 0"									
2013 MW6 2"									
2013 MW6 4'									
2013 MW6 6'									
2013 MW6 8"									
2013 MW6 10"									
2015 MW4 0"									

2015 MW4 4"									
2015 MW4 6"									
2015 MW4 8"									
2015 MW4 10"									
2015 BW4 0"									
2015 BW4 2"									
2015 BW4 4"									
2015 BW4 10"									
2015 MW6 0"	4.06E-08			8.2E-09			8.920		
2015 MW6 2'									
2015 MW6 4"									
2015 MW6 6"	7.45E-08	3.89E-08	1.14E-07	1.5E-08	7.9E-09	2.3E-08	16.368	8.546	25.046
2015 MW6 8"									
2015 MW6 10"			1.02E-07			2.1E-08			22.410

Appendix D7: qPCR raw data- flow-through columns-*Dehalococcoides* and RDase gene targets

Rep	R	Sample Name	Target	Amount SYBR [ng/uL]	ng/g	**COPY No./g**=(conc ng/g*6.0221x10^23 molec/mol) / (ampl length*660 g/mol*1x10^9 ng/g)	>H2O?
1	1	R1 TOP2	*Dhc*	3.35E-05	2.68E-04	3.40E+06	3.40E+06
2	1	R1 TOP2	*Dhc*	3.14E-05	2.51E-04	3.18E+06	3.18E+06
3	1	R1 TOP2	*Dhc*	3.01E-05	2.41E-04	3.05E+06	3.05E+06
1	1	R1 MID2	*Dhc*	2.67E-05	2.14E-04	2.71E+06	2.71E+06
2	1	R1 MID2	*Dhc*	2.53E-05	2.02E-04	2.56E+06	2.56E+06
3	1	R1 MID2	*Dhc*	2.45E-05	1.96E-04	2.48E+06	2.48E+06
1	1	R1 EXIT2	*Dhc*	2.42E-05	1.94E-04	2.45E+06	2.45E+06
2	1	R1 EXIT2	*Dhc*	2.35E-05	1.88E-04	2.38E+06	2.38E+06
3	1	R1 EXIT2	*Dhc*	2.35E-05	1.88E-04	2.38E+06	2.38E+06
1	2	R2 TOP2	*Dhc*	1.74E-05	1.39E-04	1.76E+06	1.76E+06
2	2	R2 TOP2	*Dhc*	1.56E-05	1.25E-04	1.58E+06	1.58E+06
3	2	R2 TOP2	*Dhc*	1.63E-05	1.30E-04	1.65E+06	1.65E+06
1	2	R2 MID2	*Dhc*	1.12E-05	8.96E-05	1.14E+06	1.14E+06
2	2	R2 MID2	*Dhc*	1.16E-05	9.28E-05	1.18E+06	1.18E+06
3	2	R2 MID2	*Dhc*	1.10E-05	8.80E-05	1.12E+06	1.12E+06
1	2	R2 EXIT2	*Dhc*	1.56E-05	1.25E-04	1.58E+06	1.58E+06
2	2	R2 EXIT2	*Dhc*	1.50E-05	1.20E-04	1.52E+06	1.52E+06
3	2	R2 EXIT2	*Dhc*	1.47E-05	1.18E-04	1.49E+06	1.49E+06
1	3	R3 TOP2	*Dhc*	2.32E-05	1.86E-04	2.35E+06	2.35E+06
2	3	R3 TOP2	*Dhc*	2.37E-05	1.90E-04	2.40E+06	2.40E+06
3	3	R3 TOP2	*Dhc*	2.25E-05	1.80E-04	2.28E+06	2.28E+06
1	3	R3 MID2	*Dhc*	1.69E-05	1.35E-04	1.71E+06	1.71E+06
2	3	R3 MID2	*Dhc*	1.62E-05	1.30E-04	1.64E+06	1.64E+06
3	3	R3 MID2	*Dhc*	1.63E-05	1.30E-04	1.65E+06	1.65E+06
1	3	R3 EXIT2	*Dhc*	1.46E-05	1.17E-04	1.48E+06	1.48E+06
2	3	R3 EXIT2	*Dhc*	1.43E-05	1.14E-04	1.45E+06	1.45E+06
3	3	R3 EXIT2	*Dhc*	1.44E-05	1.15E-04	1.46E+06	1.46E+06
1	4	R4 TOP2	*Dhc*	2.62E-05	2.10E-04	2.66E+06	2.66E+06
2	4	R4 TOP2	*Dhc*	2.63E-05	2.10E-04	2.67E+06	2.67E+06
3	4	R4 TOP2	*Dhc*	2.58E-05	2.06E-04	2.62E+06	2.62E+06
1	4	R4 MID2	*Dhc*	1.53E-05	1.22E-04	1.55E+06	1.55E+06
2	4	R4 MID2	*Dhc*	1.71E-05	1.37E-04	1.73E+06	1.73E+06
3	4	R4 MID2	*Dhc*	1.73E-05	1.38E-04	1.75E+06	1.75E+06
1	4	R4 EXIT2	*Dhc*	1.80E-05	1.44E-04	1.82E+06	1.82E+06
2	4	R4 EXIT2	*Dhc*	1.70E-05	1.36E-04	1.72E+06	1.72E+06
3	4	R4 EXIT2	*Dhc*	1.71E-05	1.37E-04	1.73E+06	1.73E+06
		Dhc 1	*Dhc*	3.70E-02	2.96E-01	3.75E+09	3.75E+09
		Dhc 1	*Dhc*	3.70E-03	2.96E-02	3.75E+08	3.75E+08
		Dhc 1	*Dhc*	3.70E-04	2.96E-03	3.75E+07	3.75E+07
		Dhc 1	*Dhc*	3.70E-05	2.96E-04	3.75E+06	3.75E+06
		Dhc 1	*Dhc*	3.70E-06	2.96E-05	3.75E+05	3.75E+05

		Dhc 1	*Dhc*	3.70E-07	2.96E-06		3.75E+04	3.75E+04
		Dhc 1	*Dhc*	3.70E-08	2.96E-07		3.75E+03	3.75E+03
		Dhc 1	*Dhc*	0.37	2.96E+00		3.75E+10	3.75E+10
		Dhc 1	*Dhc*	3.70E-02	2.96E-01		3.75E+09	3.75E+09
		Dhc 1	*Dhc*	3.70E-03	2.96E-02		3.75E+08	3.75E+08
		Dhc 1	*Dhc*	3.70E-04	2.96E-03		3.75E+07	3.75E+07
		Dhc 1	*Dhc*	3.70E-05	2.96E-04		3.75E+06	3.75E+06
		Dhc 1	*Dhc*	3.70E-06	2.96E-05		3.75E+05	3.75E+05
		Dhc 1	*Dhc*	3.70E-07	2.96E-06		3.75E+04	3.75E+04
		Dhc 1	*Dhc*	3.70E-08	2.96E-07		3.75E+03	3.75E+03
		Dhc 1	*Dhc*	0.37	2.96E+00		3.75E+10	3.75E+10
		Dhc 1	*Dhc*	3.70E-02	2.96E-01		3.75E+09	3.75E+09
		Dhc 1	*Dhc*	3.70E-03	2.96E-02		3.75E+08	3.75E+08
		Dhc 1	*Dhc*	3.70E-04	2.96E-03		3.75E+07	3.75E+07
		Dhc 1	*Dhc*	3.70E-05	2.96E-04		3.75E+06	3.75E+06
		Dhc 1	*Dhc*	3.70E-06	2.96E-05		3.75E+05	3.75E+05
		Dhc 1	*Dhc*	3.70E-07	2.96E-06		3.75E+04	3.75E+04
		Dhc 1	*Dhc*	3.70E-08	2.96E-07		3.75E+03	3.75E+03
		Dhc (1)	*Dhc*	3.70E-05	2.96E-04		3.75E+06	3.75E+06
		Dhc (1)	*Dhc*	3.70E-05	2.96E-04		3.75E+06	3.75E+06
		Dhc (1)	*Dhc*	3.70E-05	2.96E-04		3.75E+06	3.75E+06
		H2O	*Dhc*	2.17E-09	1.74E-08		**2.20E+02**	
		H2O	*Dhc*	-				
		H2O	*Dhc*	-				
1	4	R4 EXIT2	*tceA*	2.50E-08	2.00E-07		2.72E+03	2.72E+03
2	4	R4 EXIT2	*tceA*	2.59E-08	2.07E-07		2.83E+03	2.83E+03
3	4	R4 EXIT2	*tceA*	2.16E-08	1.72E-07		2.35E+03	2.35E+03
1	4	R4 MID2	*tceA*	5.31E-09	4.24E-08		5.78E+02	5.78E+02
2	4	R4 MID2	*tceA*	7.29E-09	5.83E-08		7.94E+02	7.94E+02
3	4	R4 MID2	*tceA*	3.98E-09	3.18E-08		4.34E+02	4.34E+02
1	4	R4 TOP2	*tceA*	1.85E-08	1.48E-07		2.01E+03	2.01E+03
2	4	R4 TOP2	*tceA*	1.74E-08	1.39E-07		1.89E+03	1.89E+03
3	4	R4 TOP2	*tceA*	1.68E-08	1.35E-07		1.83E+03	1.83E+03
1	3	R3 EXIT2	*tceA*	4.52E-07	3.62E-06		4.93E+04	4.93E+04
2	3	R3 EXIT2	*tceA*	4.51E-07	3.61E-06		4.92E+04	4.92E+04
3	3	R3 EXIT2	*tceA*	4.37E-07	3.50E-06		4.77E+04	4.77E+04
1	3	R3 MID2	*tceA*	N/A	#VALUE!	#VALUE!		#VALUE!
2	3	R3 MID2	*tceA*	4.40E-07	3.52E-06		4.80E+04	4.80E+04
3	3	R3 MID2	*tceA*	4.29E-07	3.43E-06		4.67E+04	4.67E+04
1	3	R3 TOP2	*tceA*	3.86E-07	3.08E-06		4.20E+04	4.20E+04
2	3	R3 TOP2	*tceA*	3.85E-07	3.08E-06		4.20E+04	4.20E+04
3	3	R3 TOP2	*tceA*	3.65E-07	2.92E-06		3.97E+04	3.97E+04
1	2	R2 EXIT2	*tceA*	3.76E-08	3.01E-07		4.10E+03	4.10E+03
2	2	R2 EXIT2	*tceA*	3.94E-08	3.15E-07		4.29E+03	4.29E+03
3	2	R2 EXIT2	*tceA*	3.80E-08	3.04E-07		4.13E+03	4.13E+03
1	2	R2 MID2	*tceA*	3.04E-08	2.43E-07		3.31E+03	3.31E+03

2	2	R2 MID2	*tceA*	2.63E-08	2.10E-07	2.86E+03	2.86E+03
3	2	R2 MID2	*tceA*	2.89E-08	2.31E-07	3.15E+03	3.15E+03
1	2	R2 TOP2	*tceA*	1.69E-08	1.35E-07	1.84E+03	1.84E+03
2	2	R2 TOP2	*tceA*	1.34E-08	1.07E-07	1.46E+03	1.46E+03
3	2	R2 TOP2	*tceA*	1.76E-08	1.41E-07	1.92E+03	1.92E+03
1	1	R1 EXIT2	*tceA*	9.18E-08	7.35E-07	1.00E+04	1.00E+04
2	1	R1 EXIT2	*tceA*	9.41E-08	7.53E-07	1.03E+04	1.03E+04
3	1	R1 EXIT2	*tceA*	9.53E-08	7.62E-07	1.04E+04	1.04E+04
1	1	R1 MID2	*tceA*	1.70E-07	1.36E-06	1.85E+04	1.85E+04
2	1	R1 MID2	*tceA*	1.62E-07	1.29E-06	1.76E+04	1.76E+04
3	1	R1 MID2	*tceA*	1.67E-07	1.34E-06	1.82E+04	1.82E+04
1	1	R1 TOP2	*tceA*	9.18E-08	7.35E-07	1.00E+04	1.00E+04
2	1	R1 TOP2	*tceA*	9.96E-08	7.97E-07	1.09E+04	1.09E+04
3	1	R1 TOP2	*tceA*	9.84E-08	7.87E-07	1.07E+04	1.07E+04
		Std-09	*tceA*	4.40E-08	3.52E-07	4.79E+03	4.79E+03
		Std-08	*tceA*	4.40E-07	3.52E-06	4.79E+04	4.79E+04
		Std-08	*tceA*	4.40E-07	3.52E-06	4.79E+04	4.79E+04
		Std-08	*tceA*	4.40E-07	3.52E-06	4.79E+04	4.79E+04
		Std-07	*tceA*	4.40E-06	3.52E-05	4.79E+05	4.79E+05
		Std-07	*tceA*	4.40E-06	3.52E-05	4.79E+05	4.79E+05
		Std-07	*tceA*	4.40E-06	3.52E-05	4.79E+05	4.79E+05
		Std-06	*tceA*	4.40E-05	3.52E-04	4.79E+06	4.79E+06
		Std-06	*tceA*	4.40E-05	3.52E-04	4.79E+06	4.79E+06
		Std-06	*tceA*	4.40E-05	3.52E-04	4.79E+06	4.79E+06
		Std-05	*tceA*	4.40E-04	3.52E-03	4.79E+07	4.79E+07
		Std-05	*tceA*	4.40E-04	3.52E-03	4.79E+07	4.79E+07
		Std-05	*tceA*	4.40E-04	3.52E-03	4.79E+07	4.79E+07
		Std-04	*tceA*	4.40E-03	3.52E-02	4.79E+08	4.79E+08
		Std-04	*tceA*	4.40E-03	3.52E-02	4.79E+08	4.79E+08
		Std-04	*tceA*	4.40E-03	3.52E-02	4.79E+08	4.79E+08
		Std-03	*tceA*	4.40E-02	3.52E-01	4.79E+09	4.79E+09
		Std-03	*tceA*	4.40E-02	3.52E-01	4.79E+09	4.79E+09
		Std-03	*tceA*	4.40E-02	3.52E-01	4.79E+09	4.79E+09
		Std-02	*tceA*	4.40E-01	3.52E+00	4.79E+10	4.79E+10
		Std-02	*tceA*	4.40E-01	3.52E+00	4.79E+10	4.79E+10
		Std-01	*tceA*	4.40E+00	3.52E+01	4.79E+11	4.79E+11
		Std-01	*tceA*	4.40E+00	3.52E+01	4.79E+11	4.79E+11
		Pos Ctrl	*tceA*	2.09E-05	1.67E-04	2.28E+06	2.28E+06
		Pos Ctrl	*tceA*	5.43E-05	4.34E-04	5.92E+06	5.92E+06
		Pos Ctrl	*tceA*	5.79E-05	4.63E-04	6.31E+06	6.31E+06
		Neg Ctrl	*tceA*	2.77E-10	2.21E-09	**3.02E+01**	
		Neg Ctrl	*tceA*	N/A	#VALUE!		
		Neg Ctrl	*tceA*	N/A	#VALUE!		
1	1	R1 TOP2	*vcrA*	3.39E-07	2.71E-06	3.81E+04	3.81E+04
2	1	R1 TOP2	*vcrA*	3.63E-07	2.90E-06	4.08E+04	4.08E+04
3	1	R1 TOP2	*vcrA*	3.86E-07	3.09E-06	4.33E+04	4.33E+04

1	1	R1 MID2	*vcrA*	4.76E-07	3.81E-06	5.35E+04	5.35E+04
2	1	R1 MID2	*vcrA*	4.36E-07	3.49E-06	4.90E+04	4.90E+04
3	1	R1 MID2	*vcrA*	4.35E-07	3.48E-06	4.89E+04	4.89E+04
1	1	R1 EXIT2	*vcrA*	2.99E-07	2.39E-06	3.36E+04	3.36E+04
2	1	R1 EXIT2	*vcrA*	2.87E-07	2.30E-06	3.22E+04	3.22E+04
3	1	R1 EXIT2	*vcrA*	2.75E-07	2.20E-06	3.09E+04	3.09E+04
1	2	R2 TOP2	*vcrA*	1.00E-07	8.00E-07	1.12E+04	1.12E+04
2	2	R2 TOP2	*vcrA*	7.99E-08	6.39E-07	8.97E+03	8.97E+03
3	2	R2 TOP2	*vcrA*	9.30E-08	7.44E-07	1.04E+04	1.04E+04
1	2	R2 MID2	*vcrA*	1.27E-07	1.02E-06	1.43E+04	1.43E+04
2	2	R2 MID2	*vcrA*	1.36E-07	1.09E-06	1.53E+04	1.53E+04
3	2	R2 MID2	*vcrA*	1.47E-07	1.18E-06	1.65E+04	1.65E+04
1	2	R2 EXIT2	*vcrA*	2.38E-07	1.90E-06	2.67E+04	2.67E+04
2	2	R2 EXIT2	*vcrA*	2.26E-07	1.81E-06	2.54E+04	2.54E+04
3	2	R2 EXIT2	*vcrA*	2.33E-07	1.86E-06	2.62E+04	2.62E+04
1	3	R3 TOP2	*vcrA*	9.07E-07	7.26E-06	1.02E+05	1.02E+05
2	3	R3 TOP2	*vcrA*	9.56E-07	7.65E-06	1.07E+05	1.07E+05
3	3	R3 TOP2	*vcrA*	9.12E-07	7.30E-06	1.02E+05	1.02E+05
1	3	R3 MID2	*vcrA*	1.36E-06	1.09E-05	1.53E+05	1.53E+05
2	3	R3 MID2	*vcrA*	1.16E-06	9.28E-06	1.30E+05	1.30E+05
3	3	R3 MID2	*vcrA*	1.21E-06	9.68E-06	1.36E+05	1.36E+05
1	3	R3 EXIT2	*vcrA*	1.25E-06	1.00E-05	1.40E+05	1.40E+05
2	3	R3 EXIT2	*vcrA*	1.07E-06	8.56E-06	1.20E+05	1.20E+05
3	3	R3 EXIT2	*vcrA*	1.17E-06	9.36E-06	1.31E+05	1.31E+05
1	4	R4 TOP2	*vcrA*	8.94E-08	7.15E-07	1.00E+04	1.00E+04
2	4	R4 TOP2	*vcrA*	1.83E-07	1.46E-06	2.06E+04	2.06E+04
3	4	R4 TOP2	*vcrA*	1.30E-07	1.04E-06	1.46E+04	1.46E+04
1	4	R4 MID2	*vcrA*	2.53E-08	2.02E-07	2.84E+03	2.84E+03
2	4	R4 MID2	*vcrA*	3.22E-08	2.58E-07	3.62E+03	3.62E+03
3	4	R4 MID2	*vcrA*	2.96E-08	2.37E-07	3.32E+03	3.32E+03
1	4	R4 EXIT2	*vcrA*	8.78E-08	7.02E-07	9.86E+03	9.86E+03
2	4	R4 EXIT2	*vcrA*	8.93E-08	7.14E-07	1.00E+04	1.00E+04
3	4	R4 EXIT2	*vcrA*	9.85E-08	7.88E-07	1.11E+04	1.11E+04
		vcrA 23	*vcrA*	4.8	3.84E+01	5.39E+11	5.39E+11
		vcrA 23	*vcrA*	0.48	3.84E+00	5.39E+10	5.39E+10
		vcrA 23	*vcrA*	4.80E-02	3.84E-01	5.39E+09	5.39E+09
		vcrA 23	*vcrA*	4.80E-03	3.84E-02	5.39E+08	5.39E+08
		vcrA 23	*vcrA*	4.80E-04	3.84E-03	5.39E+07	5.39E+07
		vcrA 23	*vcrA*	4.80E-05	3.84E-04	5.39E+06	5.39E+06
		vcrA 23	*vcrA*	4.80E-06	3.84E-05	5.39E+05	5.39E+05
		vcrA 23	*vcrA*	4.80E-07	3.84E-06	5.39E+04	5.39E+04
		vcrA 23	*vcrA*	4.80E-08	3.84E-07	5.39E+03	5.39E+03
		vcrA 23	*vcrA*	4.8	3.84E+01	5.39E+11	5.39E+11
		vcrA 23	*vcrA*	0.48	3.84E+00	5.39E+10	5.39E+10
		vcrA 23	*vcrA*	4.80E-02	3.84E-01	5.39E+09	5.39E+09
		vcrA 23	*vcrA*	4.80E-03	3.84E-02	5.39E+08	5.39E+08

		vcrA 23	*vcrA*	4.80E-04	3.84E-03	5.39E+07	5.39E+07
		vcrA 23	*vcrA*	4.80E-05	3.84E-04	5.39E+06	5.39E+06
		vcrA 23	*vcrA*	4.80E-06	3.84E-05	5.39E+05	5.39E+05
		vcrA 23	*vcrA*	4.80E-07	3.84E-06	5.39E+04	5.39E+04
		vcrA 23	*vcrA*	4.80E-08	3.84E-07	5.39E+03	5.39E+03
		vcrA 23	*vcrA*	4.8	3.84E+01	5.39E+11	5.39E+11
		vcrA 23	*vcrA*	0.48	3.84E+00	5.39E+10	5.39E+10
		vcrA 23	*vcrA*	4.80E-02	3.84E-01	5.39E+09	5.39E+09
		vcrA 23	*vcrA*	4.80E-03	3.84E-02	5.39E+08	5.39E+08
		vcrA 23	*vcrA*	4.80E-04	3.84E-03	5.39E+07	5.39E+07
		vcrA 23	*vcrA*	4.80E-05	3.84E-04	5.39E+06	5.39E+06
		vcrA 23	*vcrA*	4.80E-06	3.84E-05	5.39E+05	5.39E+05
		vcrA 23	*vcrA*	4.80E-07	3.84E-06	5.39E+04	5.39E+04
		vcrA 23	*vcrA*	4.80E-08	3.84E-07	5.39E+03	5.39E+03
		vcrA 23	*vcrA*	4.80E-05	3.84E-04	5.39E+06	5.39E+06
		vcrA 23	*vcrA*	4.80E-05	3.84E-04	5.39E+06	5.39E+06
		vcrA 23	*vcrA*	4.80E-05	3.84E-04	5.39E+06	5.39E+06
		H2O	*vcrA*	2.93E-09	2.34E-08	**3.29E+02**	
		H2O	*vcrA*	1.56E-09	1.25E-08	**1.75E+02**	
		H2O	*vcrA*	7.14E-10	5.71E-09	**8.02E+01**	
1	1	R1 TOP2	*bvcA*	3.86E-05	3.09E-04	3.36E+05	3.36E+05
2	1	R1 TOP2	*bvcA*	2.76E-05	2.21E-04	2.40E+05	2.40E+05
3	1	R1 TOP2	*bvcA*	3.45E-05	2.76E-04	3.00E+05	3.00E+05
1	1	R2 TOP2	*bvcA*	4.75E-05	3.80E-04	4.13E+05	4.13E+05
2	1	R2 TOP2	*bvcA*	3.36E-05	2.69E-04	2.92E+05	2.92E+05
3	1	R2 TOP2	*bvcA*	3.78E-05	3.02E-04	3.29E+05	3.29E+05
1	1	R3 TOP2	*bvcA*	1.88E-05	1.50E-04	1.64E+05	1.64E+05
2	1	R3 TOP2	*bvcA*	2.26E-05	1.81E-04	1.97E+05	1.97E+05
3	1	R3 TOP2	*bvcA*	2.15E-05	1.72E-04	1.87E+05	1.87E+05
1	2	R4 TOP2	*bvcA*	2.08E-05	1.66E-04	1.81E+05	1.81E+05
2	2	R4 TOP2	*bvcA*	2.39E-05	1.91E-04	2.08E+05	2.08E+05
3	2	R4 TOP2	*bvcA*	3.04E-05	2.43E-04	2.64E+05	2.64E+05
1	2	R1 MID2	*bvcA*	1.63E-05	1.30E-04	1.42E+05	1.42E+05
2	2	R1 MID2	*bvcA*	1.42E-05	1.14E-04	1.24E+05	1.24E+05
3	2	R1 MID2	*bvcA*	4.49E-07	3.59E-06	3.91E+03	3.91E+03
1	2	R2 MID2	*bvcA*	1.75E-05	1.40E-04	1.52E+05	1.52E+05
2	2	R2 MID2	*bvcA*	2.33E-05	1.86E-04	2.03E+05	2.03E+05
3	2	R2 MID2	*bvcA*	1.58E-05	1.26E-04	1.37E+05	1.37E+05
1	3	R3 MID2	*bvcA*	1.07E-05	8.56E-05	9.31E+04	9.31E+04
2	3	R3 MID2	*bvcA*	9.33E-06	7.46E-05	8.12E+04	8.12E+04
3	3	R3 MID2	*bvcA*	1.62E-05	1.30E-04	1.41E+05	1.41E+05
1	3	R4 MID2	*bvcA*	1.48E-05	1.18E-04	1.29E+05	1.29E+05
2	3	R4 MID2	*bvcA*	1.74E-05	1.39E-04	1.51E+05	1.51E+05
3	3	R4 MID2	*bvcA*	2.06E-05	1.65E-04	1.79E+05	1.79E+05
1	3	R1 EXIT2	*bvcA*	1.21E-05	9.68E-05	1.05E+05	1.05E+05
2	3	R1 EXIT2	*bvcA*	3.47E-06	2.78E-05	3.02E+04	3.02E+04

3	3	R1 EXIT2	*bvcA*	1.00E-06	8.00E-06		8.70E+03	8.70E+03
1	4	R2 EXIT2	*bvcA*	4.12E-05	3.30E-04		3.58E+05	3.58E+05
2	4	R2 EXIT2	*bvcA*	3.70E-05	2.96E-04		3.22E+05	3.22E+05
3	4	R2 EXIT2	*bvcA*	3.51E-05	2.81E-04		3.05E+05	3.05E+05
1	4	R3 EXIT2	*bvcA*	8.12E-06	6.50E-05		7.06E+04	7.06E+04
2	4	R3 EXIT2	*bvcA*	7.68E-06	6.14E-05		6.68E+04	6.68E+04
3	4	R3 EXIT2	*bvcA*	8.38E-06	6.70E-05		7.29E+04	7.29E+04
1	4	R4 EXIT2	*bvcA*	2.92E-05	2.34E-04		2.54E+05	2.54E+05
2	4	R4 EXIT2	*bvcA*	2.90E-05	2.32E-04		2.52E+05	2.52E+05
3	4	R4 EXIT2	*bvcA*	1.82E-05	1.46E-04		1.58E+05	1.58E+05
		STD1	*bvcA*	2.9	2.32E+01		2.52E+10	2.52E+10
		STD2	*bvcA*	0.29	2.32E+00		2.52E+09	2.52E+09
		STD3	*bvcA*	2.90E-02	2.32E-01		2.52E+08	2.52E+08
		STD4	*bvcA*	2.90E-03	2.32E-02		2.52E+07	2.52E+07
		STD5	*bvcA*	2.90E-04	2.32E-03		2.52E+06	2.52E+06
		STD6	*bvcA*	2.90E-05	2.32E-04		2.52E+05	2.52E+05
		STD7	*bvcA*	2.90E-06	2.32E-05		2.52E+04	2.52E+04
		STD8	*bvcA*	2.90E-07	2.32E-06		2.52E+03	2.52E+03
		STD9	*bvcA*	2.90E-08	2.32E-07		2.52E+02	2.52E+02
		STD10	*bvcA*	2.90E-09	2.32E-08		2.52E+01	2.52E+01
		STD1	*bvcA*	2.9	2.32E+01		2.52E+10	2.52E+10
		STD2	*bvcA*	0.29	2.32E+00		2.52E+09	2.52E+09
		STD3	*bvcA*	2.90E-02	2.32E-01		2.52E+08	2.52E+08
		STD4	*bvcA*	2.90E-03	2.32E-02		2.52E+07	2.52E+07
		STD5	*bvcA*	2.90E-04	2.32E-03		2.52E+06	2.52E+06
		STD6	*bvcA*	2.90E-05	2.32E-04		2.52E+05	2.52E+05
		STD7	*bvcA*	2.90E-06	2.32E-05		2.52E+04	2.52E+04
		STD8	*bvcA*	2.90E-07	2.32E-06		2.52E+03	2.52E+03
		STD9	*bvcA*	2.90E-08	2.32E-07		2.52E+02	2.52E+02
		STD10	*bvcA*	2.90E-09	2.32E-08		2.52E+01	2.52E+01
		STD1	*bvcA*	2.9	2.32E+01		2.52E+10	2.52E+10
		STD2	*bvcA*	0.29	2.32E+00		2.52E+09	2.52E+09
		STD3	*bvcA*	2.90E-02	2.32E-01		2.52E+08	2.52E+08
		STD4	*bvcA*	2.90E-03	2.32E-02		2.52E+07	2.52E+07
		STD5	*bvcA*	2.90E-04	2.32E-03		2.52E+06	2.52E+06
		STD6	*bvcA*	2.90E-05	2.32E-04		2.52E+05	2.52E+05
		STD7	*bvcA*	2.90E-06	2.32E-05		2.52E+04	2.52E+04
		STD8	*bvcA*	2.90E-07	2.32E-06		2.52E+03	2.52E+03
		STD9	*bvcA*	2.90E-08	2.32E-07		2.52E+02	2.52E+02
		STD10	*bvcA*	2.90E-09	2.32E-08		2.52E+01	2.52E+01
		STD5	*bvcA*	2.90E-04	2.32E-03		2.52E+06	2.52E+06
		STD5	*bvcA*	2.90E-04	2.32E-03		2.52E+06	2.52E+06
		STD5	*bvcA*	2.90E-04	2.32E-03		2.52E+06	2.52E+06
		H2O	*bvcA*	-	#VALUE!	#VALUE!		
		H2O	*bvcA*	-	#VALUE!	#VALUE!		
		H2O	*bvcA*	-	#VALUE!	#VALUE!		

Appendix D8: qPCR data- flow-through columns, RDase gene ratios

		Dhc			tceA			vcrA			bvcA		
		avg	SD	N	avg	SD	N	avg	SD	N	avg	SD	N
-Fe	GW	**2.73E+06**	127418.6	9	1.72E+03	3.02E+02	9.00E+00	4.11E+04	2.71E+03	9.00E+00	1.43E+05	41370.29	9
-Fe	RAMM	1.45E+06	79425.02	9	4.56E+04	1.33E+03	8.00E+00	1.72E+04	2.36E+03	9.00E+00	2.79E+05	31503.14	9
+Fe	GW	1.82E+06	133553.1	9	3.01E+03	3.57E+02	9.00E+00	1.25E+05	6.00E+03	9.00E+00	1.19E+05	17643.1	9
+Fe	RAMM	**2.03E+06**	157390	9	1.30E+04	1.29E+03	9.00E+00	9.55E+03	1.93E+03	9.00E+00	1.97E+05	16578.75	9
		Feed											
	Iron Level	GW	RAMM		Iron	GW	RAMM	Iron	GW	RAMM	Iron	GW	RAMM
	0	2.73E+06	1.45E+06		0	1.72E+03	4.56E+04	0	4.11E+04	1.72E+04	0	1.43E+05	2.79E+05
	5	1.82E+06	2.03E+06		5	3.01E+03	1.30E+04	5	1.25E+05	9.55E+03	5	1.19E+05	1.97E+05
				R1 (-Fe/GW)	1			24			84		
				R2 (-Fe/R)	1			0.4			6		
				R3 (+Fe/GW)	1			41			40		
				R4 (+Fe/R)	1			1			15		

Bibliography

[AFCEE] Air Force Center for Engineering and the Environment (2008) Technical protocol for enhanced anaerobic bioremediation using permeable mulch biowalls and bioreactors. AFCEE Technical Directorate. May 2008. https://clu-in.org/download/techfocus/prb/Final-Biowall-Protocol-05-08.pdf

Arnold WA, Roberts AL (2000) Pathways and kinetics of chlorinated ethylene and chlorinated acetylene reaction with Fe(0) particles. Environ Sci Technol 34(9):1794–1805. https://doi.org/10.1021/ es990884q

Atashgahi S, Lu Y, Zheng Y, Saccenti E, Suarez-Diez M, Ramiro-Garcia J, Eisenmann H, Elsner M, J.M. Stams A, Springael D, Dejonghe W, Smidt H (2017) Geochemical and microbial community determinants of reductive dechlorination at a site biostimulated with glycerol. Environ Microbiol 19:968–981 . doi: 10.1111/1462-2920.13531

Chen J, Xiu Z, Lowry GV, Alvarez PJJ (2011) Effect of natural organic matter on toxicity and reactivity of nano-scale zero-valent iron. Water Res 45:1995–2001 . doi: 10.1016/j.watres.2010.11.036

Cupples AM, Spormann AM, McCarty PL (2004) Comparative evaluation of chloroethene dechlorination to ethene by *Dehalococcoides*-like microorganisms. Environ Sci Technol 38:4768–4774 . doi: 10.1021/es049965z

Diao M, Yao M (2009) Use of zero-valent iron nanoparticles in inactivating microbes. Water Res 43:5243–5251 . doi: 10.1016/j.watres.2009.08.051

Dong Y, Liang X, Krumholz LR, Philp RP, Butler EC (2009) The relative contributions of abiotic and microbial processes to the transformation of tetrachloroethylene and trichloroethylene in anaerobic microcosms. Environ Sci Technol 43:690–697 . doi: 10.1021/es801917p

Dugat-Bony E, Biderre-Petit C, Jaziri F, David MM, Denonfoux J, Lyon DY, Richard JY, Curvers C, Boucher D, Vogel TM, Peyretaillade E, Peyret P (2012) In situ TCE degradation mediated by complex dehalorespiring communities during biostimulation processes. Microb Biotechnol 5:642–653 . doi: 10.1111/j.1751-7915.2012.00339.x

Farrell J, Kason M, Melitas N, Li T (2000) Investigation of the long-term performance of zero-valent iron for reductive dechlorination of trichloroethylene. Environ Sci Technol 34:514–521 .

[FRTR] Federal Remediation Technologies Roundtable, 2003. Abstracts of Remediation Case Studies Volume 7. U.S. EPA/National Service Center for Environmental Publications, Ohio. https://www.epa.gov/sites/production/files/2015-04/documents/abstractsvol7.pdf (accessed 09 May 2018).

Gavaskar AR (1999) Design and construction techniques for permeable reactive barriers. Journal of Hazardous Materials 68:41

Gilbert O, Cortina JL, de Pablo J, Ayora C (2013) Performance of a fieldscale permeable reactive barrier based on organic substrate and zerovalent iron for in situ remediation of acidmine drainage. Environ Sci Pollut Res 20(11):7854–7862. https://doi.org/10.1007/s11356-013-1507-2

[GSI] Groundwater Services, Inc. (2004). Report for full-scale mulch wall treatment of chlorinated hydrocarbon-impacted groundwater. Groundwater Services, Inc., Texas. Contract number F41624-97-C-8020. DTIC: ADA422621. https://clu-in.org/download/contaminantfocus/dnapl/Treatment_Technologies/Mulch_Biowall.pdf (accessed 09 May 2018).

Gu B, Phelps TJ, Liang L, Dickey MJ, Roh Y, Kinsall BL, Palumbo AV, Jacobs GK (1999) Biogeochemical dynamics in zero-valent iron columns: Implication for permeable reactive barriers. Environ Sci Technol 33:2170–2177

Haest PJ, Springael D, Smolders E (2010) Dechlorination kinetics of TCE at toxic TCE concentrations: Assessment of different models. Water Res 44:331–339 . doi: 10.1016/j.watres.2009.09.033

He J, Sung Y, Krajmalnik-Brown R, Ritalahti KM, Löffler FE (2005) Isolation and characterization of Dehalococcoides sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. Environ Microbiol 7:1442–1450 . doi: 10.1111/j.1462-2920.2005.00830.x

He YT, Wilson JT, Wilkin RT (2008) Transformation of reactive iron minerals ina permeable reactive barrier (biowall) used to treat TCE in groundwater. Environ Sci Technol 42:6690-6696 https://doi.org/10.1021/es8010354

Hendrickson ER, Payne JA, Young RM, Starr MG, Perry MP, Fahnestock S, Ellis DE, Ebersole RC (2002) Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. Appl Environ Microbiol 68:485–95 . doi: 10.1128/AEM.68.2.485-495.2002

Hughes L, Robertson S (2002) Glycerol: SIDS initial assesment report for SIAM 14. United Nations Environmental Programme Publications

Ingram DT, Callahan MT, Ferguson S, Hoover DG, Chiu PC, Shelton DR, Millner PD, Camp MJ, Patel JR, Kniel KE, Sharma M (2012) Use of zero-valent iron biosand filters to reduce Escherichia coli O157:H12 in irrigation water applied to spinach plants in a field setting. J Appl Microbiol 112(3):551–560. https://doi.org/10.1111/ j.1365-2672.2011.05217.x

Kang JW, Doty SL (2014) Cometabolic degradation of trichloroethylene by *Burkholderia cepacia* G4 with poplar leaf homogenate. Canadian Journal of Microbiology 60(7):487. https://doi.org/10.1139/cjm-2014-0095

Lee PKH, Johnson DR, Holmes VF, He J, Alvarez-Cohen L (2006) Reductive dehalogenase gene expression as a biomarker for physiological activity of *Dehalococcoides* spp. Appl Environ Microbiol 72:6161–6168 . doi: 10.1128/AEM.01070-06

Lee PKH, Macbeth TW, Sorenson KS, Deeb RA, Alvarez-Cohen L (2008) Quantifying genes and transcripts to assess the in situ physiology of "*Dehalococcoides*" spp. in a trichloroethene-contaminated groundwater site. Appl Environ Microbiol 74:2728–2739 . doi: 10.1128/AEM.02199-07

Löffler FE, Yan J, Ritalahti KM, Adrian L, Edwards EA, Konstantinidis KT, Müller JA, Fullerton H, Zinder SH, Spormann AM (2013) *Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial

class, *Dehalococcoidia* classis nov., order *Dehalococcoidales* ord. nov. and famil. Int J Syst Evol Microbiol 63:625–635 . doi: 10.1099/ijs.0.034926-0
Lu, X., Wilson, J.T., Shen, H., Henry, B.M., Kampbell, D.H., 2008. Remediation of TCE-contaminated groundwater by a permeable reactive barrier filled with plant mulch (biowall). J. Environ. Sci. Heal. A 43, 24-35. https://doi.org/10.1080/10934520701750421
Magnuson JK, Stern RV, Gossett JM, Zinder SH, Burris DR (1998) Reductive dechlorination of tetrachloroethene to ethene by a two-component enzyme pathway. Applied and Environmental Microbiology 64:1270
Maymó-Gatell X, Chien YT, Gossett JM, Zinder SH (1997) Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 276:1568–1571 . doi: 10.1126/science.276.5318.1568
[NATO/CCMS] North Atlantic Treaty Organization/Committee on the Challenges of Modern Society (2001) Evaluation of demonstrated and emerging technologies for the treatment of contaminated land and groundwater (Phase III), 2000 Annual Report. EPA 542-R-01-001.
Orth WS, Gillham RW (1996) Dechlorination of trichloroethene in aqueous solution using Fe 0. Environ Sci Technol 30:66–71 . doi: 10.1021/es950053u
Paliy O (2013) The golden age of molecular ecology. Journal of Phylogenetic & Evolutionary Biology 1(3):e105
Pant P, Pant S (2010) A review: advances in microbial remediation of trichloroethylene (TCE). J Environ Sci 22(1):116–126. https://doi. org/10.1016/S1001-0742(09)60082-6
Phillips DH, Van Nooten T, Bastiaens L, Russell MI, Dickson K, Plant S, Ahad JME, Newton T, Elliot T, Kalin RM (2010) Ten year performance evaluation of a field-scale zero-valent iron permeable reactive barrier installed to remediate trichloroethene contaminated groundwater. Environ Sci Technol 44(10):3861–3869. https://doi. org/10.1021/es902737t
Rahm BG, Chauhan S, Holmes VF, Macbeth TW, Jr. Sorenson KS, Alvarez-Cohen L (2006) Molecular characterization of microbial populations at two sites with differing reductive dechlorination abilities. Biodegradation 17:523–534 . doi: 10.1007/s10532-005-9023-9
Schäfer, D., Köber, R., & Dahmke, A. (2003). Competing TCE and cis-DCE degradation kinetics by zero-valent iron- experimental results and numerical simulation. Journal of Contaminant Hydrology, 65, 183-202.
Scherer, M. M., & Tratnyek, P. G. (1995). Dechlorination of carbon tetrachloride by iron metal: Effect of reactant concentrations. 209th National Meeting, Anaheim, California, American Chemical Society, Division of Environmental Chemistry, Preprint Extended Abstracts, 35, pp. 805-806.
Seshadri R, Adrian L, Fouts DE, Eisen JA, Phillippy AM, Methe BA, Ward NL, Nelson WC, Deboy RT, Khouri HM, Kolonay JF, Dodson RJ, Daugherty SC, Brinkac LM, Sullivan SA, Madupu R, Nelson KE, Kang KH, Impraim M, Tran K, Robinson JM, Forberger HA, Fraser CM, Zinder SH, Heidelberg JF (2005) Genome sequence of the PCE-dechlorinating bacterium *Dehalococcoides ethenogenes*. Science 307:105–108 . doi: 10.1126/science.1102226

Sung Y, Ritalahti KM, Apkarian RP, Löffler FE, Lo FE (2006) Quantitative PCR confirms purity of strain GT , a novel trichloroethene-to-ethene-respiring *Dehalococcoides* isolate. Appl Environ Microbiol 72:1980 . doi: 10.1128/AEM.72.3.1980

Tan HW, Abdul Aziz AR, Aroua MK (2013) Glycerol production and its applications as a raw material: A review. Renewable and Sustainable Energy Reviews 27:118. https://doi.org/10.1016/j.rser.2013.06.035

US EPA (2001) Sources, emission and exposure to trichloroethylene (TCE) and related chemicals. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington Office, Washington, DC EPA/600/R-00/099. https://cfpub.epa.gov/ncea/risk/recordisplay. cfm?deid=21006. Accessed 12 May 2017

US EPA (2009) National primary drinking water regulations. U.S. Environmental Protection Agency, EPA/816/F/09/004. https:// www.epa.gov/sites/production/files/2016-06/documents/npwdr_ complete_table.pdf. Accessed 12 May 2017

Vainberg S, Condee CW, Steffan RJ (2009) Large-scale production of bacterial consortia for remediation of chlorinated solvent-contaminated groundwater. J Ind Microbiol Biotechnol 36:1189-1197.

Vogan JL, Focht RM, Clark DK, Graham SL (1999) Performance evaluation of a permeable reactive barrier for remediation of dissolved chlorinated solvents in groundwater. J Hazard Mater 68(1-2):97. https://doi.org/10.1016/S0304-3894(99)00033-3

Wiedemeier TH, Newell CJ, Rifai HS, Wilson JT (1996) Natural attenuation of fuels and chlorinated solvents in the subsurface. John Wiley & Sons, New York, pp 617. https://doi.org/10.1002/9780470172964

Wilkin RT, Puls RW, Sewell GW (2003) Long-term performance of permeable reactive barriers using zero-valent iron: geochemical and microbiological effects. Ground Water 41(4):493–503. https://doi. org/10.1111/j.1745-6584.2003.tb02383.x

Wüst WF, Köber R, Schlicker O, Dahmke A (1999) Combined zero- and first-order kinetic model of the degradation of TCE and cis-DCE with commercial iron. Environmental Science & Technology 33(23):4304

Yan J, Şimşir B, Farmer AT, Bi M, Yang Y, Campagna SR, Löffler FE (2016) The corrinoid cofactor of reductive dehalogenases affects dechlorination rates and extents in organohalide-respiring *Dehalococcoides mccartyi*. The ISME Journal 10(5):1092. https://doi.org/10.1038/ismej.2015.197

Yi S, Seth EC, Mien Y-J, Stabler SP, Allen RH, Alvarez-Cohen L, Taga ME (2012) Versatility in corrinoid salvaging and remodeling pathways supports corrinoid-dependent metabolism in *Dehalococcoides mccartyi*. Applied and Environmental Microbiology 78(21):7745

Zhang Y, Hu M, Li P, Wang X, Meng Q (2015a) Analysis of trichloroethylene removal and bacterial community function based on pH- adjusted in an upflow anaerobic sludge blanket reactor. Appl Microbiol Biotechnol 99(21):9289–9297. https://doi.org/10.1007/ s00253-015-6800-1

Zhang Y, Hu M, Li P, Xin W, Meng Q (2015b) Trichloroethylene removal and bacterial variations in the up-flow anaerobic sludge blanket reactor in response to temperature shifts. Applied Microbiology and Biotechnology 99(14):6091

Zhou J, Xia B, Treves DS, Wu L-Y, Marsh TL, O'Neill RV, Palumbo AV, Tiedje JM (2002) Spatial and Resource Factors Influencing High Microbial Diversity in Soil. Applied and Environmental Microbiology 68(1):326